(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 350 287 B1

(12) EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent:
27.09.2000 Bulletin 2000/39

(51) Int. Cl.[7]: **C07H 19/073**, C07F 9/6558, C07F 9/6561, C07H 19/173, A61K 9/127, A61K 31/70

(21) Application number: **89306815.5**

(22) Date of filing: **05.07.1989**

(54) **Lipid derivatives of antiviral nucleosides, liposomal incorporation and method of use**

Lipid-Derivate von antiviralen Nukleosiden, liposomale Inkorporation und Verwendungsverfahren

Dérivés lipidiques de nucléosides antiviraux, incorporation liposomale et méthode d'utilisation

(84) Designated Contracting States:
AT BE CH DE ES FR GB GR IT LI LU NL SE

(30) Priority: **07.07.1988 US 216412**
06.03.1989 US 319485
28.06.1989 US 373088

(43) Date of publication of application:
10.01.1990 Bulletin 1990/02

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Description

[0001] The present invention relates generally to the treatment of viral infections using lipid derivatives of antiviral nucleoside analogues. More particularly, the present invention relates to lipid, and especially phospholipid, derivatives of modified antiviral nucleoside analogues which can be integrated into the structure of liposomes, thereby forming a more stable liposomal complex which can deliver greater amounts of drugs to target cells with less toxicity.

[0002] The publications referred to herein are hereby incorporated by reference to the extent permitted by law.

[0003] There has been a great deal of interest in recent years in the use of nucleoside analogues to treat viral infections. A nucleoside consists of a pyrimidine or purine base which is linked to ribose, a five-carbon sugar having a cyclic structure. The antiviral nucleoside analogues closely resemble natural nucleosides and are designed to inhibit viral functions by preventing the synthesis of new DNA or RNA. Nucleosides are enzymatically assembled into DNA or RNA.

[0004] During DNA synthesis, free nucleoside triphosphates (nucleosides with three phosphate groups attached) react with the end of a growing DNA chain. The reaction involves the linking of the phosphate group at the 5′ position on the incoming nucleoside triphosphate with the hydroxyl group at the 3′ position of the sugar ring on the end of the forming DNA chain. The other two phosphate groups are freed during the reaction, thereby resulting in the addition of a nucleotide to the DNA chain.

[0005] Nucleoside analogues are compounds which mimic the naturally occurring nucleosides sufficiently so that they are able to participate in viral DNA synthesis. However, the antiviral nucleoside analogues have strategically located differences in chemical structure which inhibit viral enzymes such as reverse transcriptase or which prevent further DNA synthesis once the analogue has been attached to the growing DNA chain.

[0006] Dideoxynucleosides are antiviral compounds that lack the hydroxyl groups normally present at the second and third position of ribose. When a dideoxynucleoside is incorporated into a growing DNA chain, the absence of the 3-OH group on its ribose group makes it impossible to attach another nucleotide and the chain is terminated. Dideoxynucleosides are particularly useful in treating retroviral infections where viral replication requires the transcription of viral RNA into DNA by viral reverse transcriptase. Other nucleoside analogues include deoxynucleosides and nucleosides analogues having only a fragment of ribose or other pentose connected to the base molecule.

[0007] Acquired immunodeficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV). HIV infects cells bearing the CD4 (T4) surface antigen, such as CD4+ helper lymphocytes, CD4+ monocytes and macrophages and certain other CD4+ cell types. The HIV infection of CD4+ lymphocytes results in cytolysis and cell death which contributes to the immunodeficiency of AIDS; however, CD4+ monocytes and macrophages may not be greatly harmed by the virus. Viral replication in these cells appears to be more prolonged and less cytotoxic than in lymphocytes, and as a result, monocytes and macrophages represent important reservoirs of HIV infection. It has recently been discovered that macrophages may serve as reservoirs of HIV infection even in certain AIDS patients who test negative for the presence of HIV antibodies. No effective cure is available for AIDS, although dideoxynucleosides have been shown to prolong life and to reduce the incidence of certain fatal infections associated with AIDS.

[0008] Certain monocyte-derived macrophages, when infected with some strains of HIV, have been found to be resistant to treatment with dideoxycytidine, azidothymidine, and other dideoxynucleosides *in vitro* as shown by Richman, et al. (1). The resistance may be due in part to the low levels of dideoxynucleoside kinase which result in a reduced ability to phosphorylate AZT, ddC or ddA. Clearly, it would be useful to have more effective ways of delivering large amounts of effective antiviral compounds to macrophages infected with HIV or other viruses and other cells having viral infections. It would also be useful to have more effective ways of delivering antiviral compounds which not only increase their potency but prolong their efficacy.

[0009] Dideoxynucleoside analogues such as AZT are the most potent agents currently known for treating AIDS, but in a recent human trial, serious toxicity was noted, evidenced by anemia (24%) and granulocytopenia (16%) (2,3). It is desirable, therefore, to provide a means for administering AZT and other dideoxynucleosides in a manner such that the toxic side effects of these drugs are reduced. Further, it is desirable to provide selective targeting of the dideoxynucleoside to monocyte/macrophages to enhance the efficiency of the drug against viral infection in this group of cells. One way to do this is to take advantage of the uptake of liposomes by macrophages.

[0010] In 1965, Alex Bangham and coworkers discovered that dried films of phosphatidylcholine spontaneously formed closed bimolecular leaflet vesicles upon hydration (4). Eventually, these structures came to be known as liposomes.

[0011] A number of uses for liposomes have been proposed in medicine. Some of these uses are as carriers to deliver therapeutic agents to target organs. The agents are encapsulated during the process of liposome formation and released *in vivo* when liposomes fuse with the lipids of cell surface membrane. Liposomes provide a means of delivering higher concentrations of therapeutic agents to target organs. Further, since liposomal delivery focuses therapy at the site of liposome uptake, it reduces toxic side effects.

[0012] For example, liposomal antimonial drugs are several hundred-fold more effective than the free drug in treating leishmaniasis as shown independently by Black and Watson (5) and Alving, et al. (6). Liposome-entrapped ampho-

tericin B appears to be more effective than the free drug in treating immunosuppressed patients with systemic fungal disease (7). Other uses for liposome encapsulation include restriction of doxorubicin toxicity (8) and diminution of aminoglycoside toxicity (9).

[0013] As previously mentioned, it is now thought that macrophages are an important reservoir of HIV infection (10, 11). Macrophages are also a primary site of liposome uptake (12, 13). Accordingly, it would be desirable to utilize liposomes to enhance the effectiveness of antiviral nucleoside analogues in treating AIDS and other viral infections.

[0014] The use of liposomes to deliver phosphorylated dideoxynucleoside to AIDS infected cells which have become resistant to therapy has been proposed in order to bypass the low dideoxynucleoside kinase levels.

[0015] Attempts have also been made to incorporate nucleoside analogues, such as iododeoxyuridine (IUDR), acylovir (ACV) and ribavirin into liposomes for treating diseases other than AIDS. However, these attempts have not been entirely satisfactory because these relatively small water soluble nucleoside analogues tend to leak out of the liposome rapidly (14, 15), resulting in decreased targeting effectiveness. Other disadvantages include the tendency to leak out of liposomes in the presence of serum, difficulties in liposome formulation and stability, low degree of liposomal loading, and hydrolysis of liposomal dideoxynucleoside phosphates when exposed to acid hydrolases after cellular uptake of the liposomes.

[0016] Attempts have also been made to combine nucleoside analogues, such as arabinofuranosylcytosine (ara-C) and arabinofuranosyladenine (ara-A), with phospholipids in order to enhance their catabolic stability as chemotherapeutic agents in the treatment of various types of cancer (16). The resulting agents showed a decreased toxicity and increased stability over the unincorporated nucleoside analogues. However, the resulting agents exhibited poor cellular uptake (16) and poor drug absorption (17).

[0017] EP-A-0122151 (36) discloses the preparation of primary or secondary alcohol derivatives of phospholipids by means of an enzymatic transfer technique that exchanges the alcohol structural moiety of a phospholipid species for a primary or secondary alcohol through the enzymatic activity of phospholipase DM.

[0018] The phospholipid may be selected from, amongst others, a particular defined range of 1,2-O-diacyl and 1,3-O-diacyl glycerol monophosphate phospholipids and the primary alcohol may be selected from, amongst others, the nucleosides cytidine, uridine, arabinosylcytosine, adenosine, guanosine, cyclocytidine, deoxyadenosine, deoxycytidine, deoxyguanosine, deoxythymidine, deoxyuridine and inosine. However, EP-A-0122151 contains no teaching of any pharmacological advantages of such compounds and only very few of the possible individual compounds arising from the theoretically possible selections are taught or suggested.

[0019] J. Med. Chem. *25* (1982) pages 1322-1329 (Ryu et al) (37) discloses the preparation of certain particular phospholipid conjugates of ara-C and certain other nucleosides. The phospholipid-nucleoside linkage can be 3-diphosphate or monophosphate, depending on the particular conjugate being described, and in certain ones of the conjugates the nucleosides cytidine, arabinosylcytosine, arabinosyladenine and 7-deazaadenosine are present. A pharmacological activity against mouse myeloma is reported for certain conjugates. No suggestion is made of any antiviral or antiretroviral activity.

[0020] EP-A-0262876 (38) discloses the preparation of certain particular 1,2-O-diacyl and 1,2-O-dialkyl glycerol monophosphate phospholipid derivatives of nucleosides and reports that such derivatives have strong antitumor activity and "superior" solubility in aqueous medium. No suggestion is made of any antiviral or antiretroviral activity. The nucleosides 5-fluorouridine, 5-fluorocytidine, bredinin, tubercidin, arabinosyl-cytosine, arabinosyl-5-fluorocytosine, arabinosyl-5-fluorocytosine, arabinosyl-adenine, arabinosyl-thymine, 5-fluoro-2'-deoxyribo-uridine and Neplanocin-A are mentioned by way of example although only 5-fluorouridine, Neplanocin-A and arabinosyl-5-fluorocytosine are present in any of the specific compounds taught.

[0021] GB-A-2168350 (39) discloses the preparation of certain particular 1-O-alkyl-2-O-acyl glycerol diphosphate phospholipid derivatives of certain nucleosides in which the sugar is ribose, 2'-deoxyribose, arabinose or 2',2'-dihydroxyribose and the base is adenine, cytosine, 5-fluorouracil, 5-azacytosine, 6-mercaptopurine or 7-deazaadenine, and reports generally that such derivatives have anticancer (antitumor) and antiviral activity although only an antitumor activity of two compounds in lymphoid leukemic mice is supported by specific data.

[0022] In order to use nucleoside analogues incorporated into liposomes for treating viral infections more effectively, it is desirable to increase the stability of the association between the liposome and the nucleoside analogue.

[0023] In order to further enhance the effectiveness of these antiviral liposomes, it would be desirable to target the liposomes to infected cells or sites of infection. Greater specificity in liposomal delivery may be obtained by incorporating monoclonal antibodies or other ligands into the liposomes. Such ligands will target the liposomes to sites of liposome uptake capable of binding the ligands. Two different approaches for incorporating antibodies into liposomes to create immunoliposomes have been described: that of Huang and coworkers (18) involving the synthesis of palmitoyl antibody, and that of Leserman, et al. (19) involving the linkage of thiolated antibody to liposome-incorporated phosphatidylethanolamine (PE).

[0024] The methods disclosed here apply not only to dideoxynucleosides used in the treatment of AIDS and other retroviral diseases, but also to the use of antiviral nucleosides in the treatment of diseases caused by other viruses,

such as herpes simplex virus (HSV), human herpes virus 6, cytomegalovirus (CMV), hepatitis B virus, Epstein-Barr virus (EBV), and varicella zoster virus (VZV). Thus, the term "nucleoside analogues" is used herein to refer to compounds that can inhibit viral replication at various steps, including inhibition of viral reverse transcriptase or which can be incorporated into viral DNA or RNA, where they exhibit a chain-terminating function.

Summary of the Invention

[0025] The invention enables compounds and compositions to be provided for use in treating viral infections, including HIV (AIDS), herpes simplex virus (HSV), human herpes virus 6, cytomegalovirus (CMV), hepatitis B virus, Epstein-Barr virus (EBV), and varicella zoster virus (VZV). A composition may contain, in addition to a pharmaceutically acceptable carrier, a lipophilic antiviral compound prepared by chemically linking an antiviral nucleoside analogue to at least one lipid species. The antiviral nucleoside analogue is linked to the lipid through a monophosphate, diphosphate or triphosphate group. The invention, further, provides a method for incorporating such lipid derivatives of antiviral agents into liposomes for improved delivery of the antiviral agent. A liposome comprises a relatively spherical bilayer which is comprised wholly or in part of the above-described lipid derivatives of antiviral agents. The liposome may also contain pharmacologically inactive lipids. Further, the liposome may contain a ligand, such as a monoclonal antibody to a viral binding site (such as CD_4), or other binding protein. Such a ligand provides additional specificity in the delivery site of the antiviral agent. The invention provides a method for incorporating such ligands into antiviral liposomes.

[0026] The invention is defined in the appended claims.

[0027] In one preferred embodiment, the liponucleotide compound is a phosphatidyldideoxynucleoside or a dideoxynucleoside diphosphate diglyceride. In another, the lipid species may comprise at least one acyl ester, ether, or vinyl ether derivative of glycerol.

[0028] In another embodiment, the nucleoside analogue is a purine or pyrimidine linked through a β-N-glycosyl bond to a pentose residue that lacks at least one of the 2′ or 3′ carbons, but retains the 5′ carbon, and the phosphate group is bound to the 5′ carbon (i.e., what would have been the 5′ carbon in a complete pentose moiety). In another embodiment of the invention, the lipid species is an N-acyl sphingosine.

[0029] In some preferred embodiments, the acyl or alkyl groups of the lipid species, of whatever linkage, as for example ester, ether or vinyl ether, comprise 2 to 24 carbon atoms. In one variation, at least one of the acyl or alkyl groups is saturated. In another, at least one of the acyl or alkyl groups has up to six double bonds.

[0030] In still another embodiment, the lipid moiety is a glyceride and the glyceride has two acyl groups that are the same or different.

[0031] In addition to the compound, liposomes formed at least in part from the liponucleotide compounds, may further comprise phospholipids selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, phosphatidylinositol and sphingomyelin.

[0032] In one embodiment of the invention, the percentage of antiviral agent is 0.01 to 100 percent by weight of the liposome.

[0033] In another embodiment, the liposome further comprises a ligand bound to a lipid substrate. The ligand may be an antibody, such as a monoclonal antibody to a viral antigen. The viral antigen could be gp41 or gp110 of HIV, or could be any other suitable viral antigen. In one embodiment, the ligand is CD4 receptor protein, or CD4 protein itself. Alternatively, the ligand is an antibody to CD4 or a protein or other substance that binds CD4.

[0034] In one embodiment, the nucleoside analogue is a nitrogenous base which is a purine, pyrimidine, or a derivative thereof, and the pentose residue is a 2,′3′-dideoxy, 2′,3′-didehydro, azido or halo derivative of ribose, or an acyclic hydroxylated fragment of ribose. The pentose residue may thus be a 2′,3′-dideoxyribose, and the nucleoside analogue may be 2′,3′-dideoxycytidine, 2′,3′-dideoxythymidine, 2′,3′-dideoxyguanosine, 2′,3′-dideoxyadenosine, 2′,3′-dideoxyinosine, or 2,6 diaminopurine, 2′,3′-dideoxyriboside.

[0035] In another embodiment, the pentose residue is a 2′,3′-didehydroribose and the nucleoside is 2′,3′-didehydrothymidine, 2′,3′-didehydrocytidine carbocyclic, or 2′,3′-didehydroguanosine.
In still another embodiment, the pentose residue is an azide derivative of ribose, and the nucleoside is 3′-azido-3′-deoxythymidine, 3′-azido-3′-deoxyguanosine, or 2,6-diaminopurine-3-azido-2′,3′-dideoxyriboside.

[0036] In still another embodiment of the invention, the pentose residue is a halo derivative of ribose and the nucleoside is 3′-fluoro-3′-deoxythymidine, 3′-fluoro-2′,3′-dideoxyguanosine, 2′,3′-dideoxy-2′-fluoro-ara-adenosine, or 2,6-diaminopurine-3′-fluoro-2′,3′-dideoxyriboside. The invention also includes halo derivatives of the purine or pyrimidine rings, such as, for example, 2-chloro-deoxyadenosine. Alternatively, the pentose residue is an acyclic hydroxylated fragment of ribose, and the nucleoside is 9-(4,-hydroxy-1′,2′-butadienyl) adenine, 3-(4,-hydroxy-1′,2′-butadienyl) cytosine, 9-(2-phosphonylmethoxyethyl)adenine or phosphonomethoxydiaminopurine.

[0037] In accordance with another embodiment of the invention, the nucleoside analogue is acyclovir, gancyclovir, 1-(2′-deoxy-2′-fluoro-1-β-D-arabinofuranosyl)-5-iodocytosine (FIAC) or 1(2′-deoxy-2′-fluoro-1-β-D-arabinofuranosyl)-5-iodouracil (FIAU).

[0038] In all of the foregoing compounds, a monophosphate, diphosphate, or triphosphate linking group may be provided between the 5′ position of the pentose residue and the lipid species. In still further embodiments of the invention, the lipid species is a monoacylglycerol or a diacylglycerol. Other examples of lipid species include D,L-1,2-diacyloxyethyl-(dimethyl)-beta-hydroxyethyl ammonium groups.

[0039] In accordance with another embodiment of the present invention, the lipid species comprises from 1 to 4 fatty acid moieties, each the moiety comprising from 2 to 24 carbon atoms. Advantageously, at least one fatty acid moiety of the lipid species is unsaturated, and has from 1 to 6 double bonds.

[0040] A particular example of these compounds is 1,2-diacylglycerophospho-5′-(2′,3′-dideoxy)thymidine.

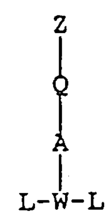
[0041] Specific examples of these compounds are provided having the formula:

$$(L)_m\text{-}(W)_n\text{-}A\text{-}Q\text{-}Z$$

wherein

Z is the base portion of the nucleoside analogue, Q is the pentose residue, A is O or S, W is phosphate, n = 1 to 3, and L is the lipid moiety wherein m = 1 to 5, and wherein each L is linked directly to a W.

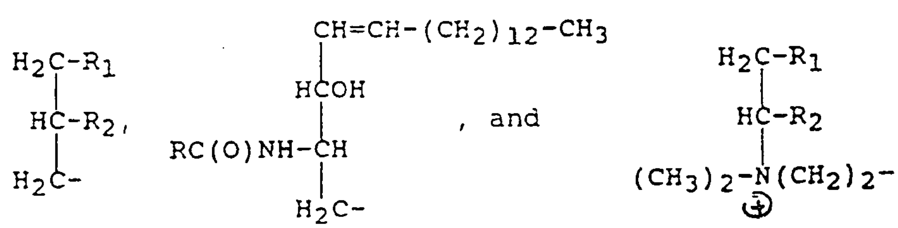
[0042] Also included are compounds having the formula:



wherein Z is the substituted or unsubstituted purine or pyrimidine group of the nucleoside analogue,

Q is the pentose residue,
W is phosphate, A is O or S, and
L is the lipid moiety.

[0043] In one embodiment of the invention, with reference to the foregoing formulas, each L is independently selected from the group consisting of



wherein R, R_1 and R_2 independently have from 0 to 6 sites of unsaturation, and have the structure

$$CH_3\text{-}(CH_2)_a\text{-}(CH{=}CH\text{-}CH_2)_b\text{-}(CH_2)_c\text{-}Y$$

wherein the sum of a and c is from 1 to 23, and b is 0 to 6, and wherein Y is C(O)O-, C-0-, C=C-O-, C(O)S-, C-S-, or C=C-S-.

[0044] In one embodiment of the foregoing compounds, the pentose residue comprises ribose, dideoxyribose, didehydroribose, or an azido or halosubstituted ribose, attached at the 9 position of the purine or at the 1 position of the pyrimidine.

[0045] A method for synthesizing the lipid derivatives of antiviral nucleosides comprises the step of reacting an antiviral nucleoside, having a ribose hydroxyl group, with a phospholipid in the presence of a coupling reagent whereby the

nucleoside is joined to the phospholipid by a phosphate bond at the position of the ribose hydroxyl group. In one preferred embodiment, the phospholipid is a diacyl phosphate. In another, the phospholipid is a phosphatidic acid or a ceramide. A further method of synthesizing the lipid derivatives of antiviral nucleosides comprises the steps of reacting an antiviral nucleoside monophosphate with a reagent HL, wherein L represents a leaving group, to form a nucleoside PO_4-L, reacting the nucleoside PO_4-L with a phosphatidic acid to bind the acid to the nucleoside through a pyrophosphate bond. In one variation of the method, the nucleoside monophosphate is AZT 5'-monophosphate.

[0046] Still a further method of synthesizing a glyceride derivative of a nucleoside analogue comprises the step of joining a monoglyceride or diglyceride and an antiviral nucleoside monophosphate with a coupling agent in the presence of a basic catalyst. In one embodiment, the glyceride is 1-O-stearoylglycerol and the nucleoside is AZT monophosphate.

[0047] A method for preparing a suspension of the liposomes for use in treating viral and retroviral infections in a mammal comprises providing the lipophilic antiviral agent comprising at least one lipid species attached to a nucleoside analogue through a monophosphate, diphosphate or triphosphate linking group at the 5' position of the pentose residue of the nucleoside, combining the lipophilic antiviral agent and a pharmacologically acceptable aqueous solvent to form a mixture, and forming liposomes from the lipophilic antiviral agent. The liposomes may be formed, for example, by sonication, extrusion or microfluidization. In one preferred embodiment, the combining step further comprises including in the combination a pharmacologically inactive lipophilic lipid. This inactive lipid can be, for example, a phosphatidylethanolamine, a sphingolipid, a sterol or a glycerophosphatide. The method also may include treating the liposomes with thio-antibodies to produce immunoliposomes, or including in the combination an lipophilic lipid which is, in part, comprised of a ligand. Thus, the liposome may include a ligand bound to a lipid substrate.

[0048] The invention enables a method for treating retroviral and viral infections in a mammal, such as a human, by administering a sufficient quantity of the antiviral nucleoside analogues described herein to deliver a therapeutic dose of the antiviral agent to the mammal. In a preferred embodiment, the method is used to treat retroviral and viral infections in a mammal, wherein the retrovirus has become resistant to therapy with conventional forms of an antiviral agent. The present invention also enables a method for treatment of patients having strains of HIV that have developed resistance to AZT or reduced sensitivity to AZT, comprising the step of administering a compound of the present invention to such patient in an effective, retrovirus-inhibiting dosage. Also enabled by the present invention is a method for treating a viral infection in a mammal, comprising the step of administering an effective amount of a compound as described herein to a mammal. The infection may be a herpes simplex infection, and the compound may be phosphatidylacyclovir. Alternatively, the virus may be HIV retrovirus, and the compound may be 5'-palmitoylAZT. The method includes use where the retrovirus is a strain of HIV that has developed resistance to a nucleoside analogue.

[0049] Also enabled by the present invention is a method for prolonging the antiviral effect of a nucleoside analogue in a mammal, comprising administering the nucleoside analogue to the mammal in the form of the nucleoside-lipid derivatives disclosed herein. Also enabled is a method for avoiding or overcoming resistance of the retrovirus to nucleoside analogues through administering the analogue in the form of the lipid derivative compounds disclosed herein.

[0050] The present invention enables use of the compounds and compositions of the invention in the preparation of a medicament for treatment of a human viral infection. The compositions of the invention may comprise a compound of the invention and a pharmaceutically acceptable carrier. Compositions of the invention may comprise a compound of the invention and at least one other antiviral compound.

[0051] Liposomal delivery of antiretroviral and antiviral drugs results in higher dosing of macrophage and monocyte cells which take up liposomes readily. The unique advantages of the present invention are that the lipid derivatives of the antiviral nucleosides are incorporated predominantly into the phospholipid layer of the liposome rather than in the aqueous compartment. This allows larger quantities of antiviral analogue to be incorporated in liposomes than is the case when water soluble phosphate esters of the nucleosides are used. Complete incorporation of the antiviral derivative into liposomes will be obtained, thus improving both the drug to lipid ratio and the efficiency of formulation. Further, there will be no leakage of the antiviral lipid analogues from the liposome during storage. Finally, liposomal therapy using these compounds allows larger amounts of antiviral compound to be delivered to the infected macrophage and monocyte cells. Therapy with liposomal compounds containing site specific ligands allows still greater amounts of antiviral compounds to be delivered with increased specificity.

[0052] Another novel advantage of this invention is that each class of lipid derivatives of antiviral nucleosides disclosed below is believed to give rise directly to antiviral phosphorylated or non-phosphorylated nucleosides upon cellular metabolism.

[0053] A further advantage of this invention is that the novel lipid derivatives are incorporated into the cell, protecting the cell for prolonged periods of time, up to or exceeding 48 hours after the drug is removed.

[0054] These and other advantages and features of the present invention will become more fully apparent from the following description and appended claims.

Brief Description of the Drawings

[0055] Figures 1-5 are graphs plotting p24 production by HIV-infected cells as a function of the amount of the compound of the present invention administered *in vitro*.

Detailed Description of the Invention

[0056] The present invention involves lipid derivatives of nucleoside analogues which can be incorporated into the lipid bilayer of liposomes. These derivatives are converted into nucleoside analogues by constituent cellular metabolic processes, and have antiviral effects *in vivo* and *in vitro*.

[0057] Suitable lipid derivatives of nucleoside analogues comprise phosphatidyl nucleosides, nucleoside diphosphate diacylglycerols, and ceramide phosphonucleosides. The lipid derivatives of these compounds provide one or two hydrophobic acyl groups to anchor the nucleoside in the lipid bilayer of the liposome. The present invention also enables lipid derivatives capable of providing additional acyl groups, and hence greater anchoring strength for nucleoside analogues. The increase in anchoring strength makes it possible to utilize nucleoside analogues of greater polarity in liposome formulations. Accordingly we disclose additional nucleoside structures of this type for use in liposomal therapies.

Nomenclature:

[0058] The lipid derivatives of the present invention are made up of complex structures which can only be rigorously defined by cumbersome terminology. For purposes of clarity, the descriptions of lipid and nucleosides components and their combinations will be in terms of commonly used trivial names, familiar to those in the art. For example , the well known drug, 3′-azido-3′-deoxythymidine, will be frequently referred to as AZT. Similarly the derivative of AZT comprising a 1,2 diacylglycerol-3-phosphate moiety, will be frequently referred to as phosphatidylAZT or pAZT. Parallel derivatives of dideoxythymidine or dideoxycytidine will correspondingly be referred to as phosphatidylddT or pddT and phosphatidylddC and pddC. Derivatives of halogenated nucleosides will be referred to as, for example, phosphatidyl-3′BrddT.

[0059] The nucleoside analogues for use in the invention can be any nucleoside that does not occur naturally in the species to be treated for viral infection. It may comprise a naturally occurring purine or pyrimidine base attached to an analogue of a naturally occurring ribose group. It may likewise comprise an analogue of a purine or pyrimidine base attached to a ribose or deoxyribose group which is present in naturally occurring nucleosides. Alternatively, both the base and the ribose moieties of the nucleoside analogues may be analogues of those found in nature. A nucleoside analogue may also comprise either a normal base or a base analogue attached to a non-ribose sugar moiety.

[0060] Analogues of both the purine or pyrimidine base and the ribose group can differ from a corresponding naturally occurring moiety by having new substituent groups attached thereto, by having naturally occurring substituent groups deleted therefrom, or by having atoms normally present replaced by others. Examples of analogues formed by substitution are 2,6-diaminopurine and 3′-azido-3′deoxyribose; by deletion, 6-oxypurine or didehydroribose; by replacement, 8-azaguanine.

[0061] Nucleoside analogues may also comprise a purine or pyrimidine base attached to the pentose moiety in a non-naturally occurring linkage, such as, for example through the nitrogen at the 3 position rather than the 1 position of the pyrimidines.

[0062] In general, the nucleoside analogues used in preparing the liposomes of the present invention will have a purine or pyrimidine base, e.g., adenine, guanine, cytosine or thymine, or an analogue thereof, attached to a pentose, such as ribose or a ribose residue and/or derivative. The attachment is through the nitrogen in the 9 position of the purines and through the nitrogen in the 1 position of the pyrimidines. These nitrogens are linked by a β-N glycosyl linkage to carbon 1 of the pentose residue.

[0063] The pentose residue may be a complete pentose, or a derivative such as a deoxy- or dideoxypentose. In addition, the pentose residue can be a fragment of a pentose, such as a hydroxylated 2-propoxymethyl residue or a hydroxylated ethoxymethyl residue. Particular nucleoside residues having these structures include acyclovir and ganciclovir. The pentose may also have an oxygen or sulfur substitution for a carbon atom at, for example, the 3′position of deoxyribose (BCH-189).

[0064] The phosphate groups are generally connected to the 5′ carbon of the pentoses in the compounds of the present invention; however, compounds wherein the phosphate groups are attached to the 3′ hydroxyl group of the pentose are within the invention if they possess antiviral activity.

[0065] It is important to recognize that in compounds having pentose residues that are not complete pentoses, the phosphate groups are connected to the carbon that would have been the 5′ carbon if the pentose were complete. In these pentose fragments, the 2′ and/or 3′ carbons may be missing; nevertheless, they are considered to be nucleoside derivatives within the meaning of present invention, and the carbon atom to which the phosphate groups are connected will generally be referred to herein as the 5′ carbon for purposes of consistency of usage.

[0066] The antiviral activity of the conjugates described herein may reside in any component of the lipid-nucleoside complex, that is, in a nucleoside base analogue, in a ribose analogue, or in the substitution of another pentose for ribose. It may also reside in the complex as a whole, wherein, for example, a weakly antiviral analogue or one possessing imperceptible or latent viral activity becomes more potent following its incorporation into a lipid derivative of a nucleotide.

[0067] Nucleosides known to have such activity are members of the class comprising 3′-azido-2′,3′-dideoxypyrimidine nucleosides, for example, AZT, AZT-P-AZT, AZT-P-ddA, AZT-P-ddI, AzddClU, AzddMeC, AzddMeC N4-OH, AzddMeC N4Me, AZT-P-CyE-ddA, AzddEtU(CS-85), AzddU(CS-87), AzddC(CS-91), AzddFC, AzddBrU, and AzddIU; the class comprising 3′-halopyrimidine dideoxynucleosides, for example, 3-FddClU, 3-FddU, 3-FddT, 3-FddBrU, and 3-FddEtU; the class comprising 2′,3′-didehydro-2′,3′-dideoxynucleosides (D4 nucleosides), for example, D4T, D4C, D4MeC, and D4A; the class comprising 2′,3′-unsubstituted dideoxypyrimidine nucleosides, for example, 5-F-ddC, ddC and ddT; the class comprising 2′,3′-unsubstituted dideoxypurines nucleosides, for example, ddA, ddDAPR(diaminopurine), ddG, ddI, and ddMeA(N6 methyl); and the class comprising sugar-substituted dideoxypurine nucleosides, for example, 3-N_3ddDAPR, 3-N_3ddG, 3-FddDAPR, 3-FddG, 3-FddaraA, and 3-FddA, wherein Me is methyl, Et is ethyl and CyEt is cyanoethyl.

[0068] Other suitable nucleotide analogues may be antiviral agents like acyclovir or ganciclovir (DHPG), or other analogues, as described below. Preferred dideoxy derivatives are those used in the treatment of AIDS, including 3′-azido-3′-deoxythymidine (azidothymidine or AZT); 2′,3′-dideoxythymidine (ddT); 2′,3′-dideoxycytidine (ddC); 2′,3′-dideoxyadenosine (ddA); and 2′,3′-dideoxyguanosine (ddG). AZT, ddT, and ddC are most preferred analogues at present. The didehydropyrimidines, as well as carbovir, a carbocyclic 2′,3′-didehydroguanosine, are also preferred. The 3′-azido derivatives of deoxyguanosine (AZG) and the pyrimidine, deoxyuridine, and the 3′-fluoro derivatives of deoxythymidine and deoxyguanosine are preferred as well. Among the 2′,6′-diaminopurines, the 2′,3′-deoxyriboside and its 3′-fluoro and 3′-azido derivatives are preferred. Also preferred is 2-chloro-deoxyadenosine.

[0069] Among the acyclic sugar derivatives, 9-(4,-hydroxy-1′,2′-butadienyl)adenine (adenallene) and its cytosine equivalent are preferred. Preferred acyclic derivatives having a purine or diaminopurine base are 9-(2-phosphonylmethoxyethyl)adenine and phosphonomethoxyethyl deoxydiaminopurine (PMEDADP).

[0070] Stereoisomers of these nucleosides, such as 2′-fluoro-ara-ddA, may be advantageous because of their resistance to acid-catalyzed hydrolysis of the glycosidic bond, which prolongs their antiviral activity. In such cases, they are preferred.

[0071] For treating herpes, cytomegalovirus and hepatitis B infections, one may utilize the lipid derivatives of acyclovir, ganciclovir, 1-(2′-deoxy 2′-fluoro-1-β-D-arabinofuranosyl)-5-iodocytosine (FIAC) or 1(2′-deoxy-2′-fluoro-1-β-D-arabinofuranosyl)-5-iodouracil (FIAU).

[0072] The lipids are attached to the nucleoside analogues through phosphate linkages. Lipid derivatives comprising a phosphate link between a nucleoside analogue and lipid may be prepared from phospholipids, phosphorylated nucleoside analogs, or both. Suitable phospholipids comprise phosphoglycerides or sphingolipids.

[0073] Lipid derivatives of nucleoside analogue in which lipids are linked either through mono-, di-, or triphosphate groups may be prepared from phosphorylated nucleoside analogues. Phosphorylated nucleoside analogues are known. The dideoxynucleoside analogue is phosphorylated according to conventional procedures such as the phosphorous oxychloride method of Toorchen and Topal (20). The preferred modified analogue is the 5′-monophosphate. Since AZT, ddC and other dideoxynucleosides have only the 5′-hydroxyl, only the 5′-monophosphate is formed during phosphorylation; however, in other analogues in which the 3′hydroxyl is present, a 3′-monophosphate can be formed. The diphosphate and triphosphate analogues of antiviral nucleosides may also be used.

[0074] The aliphatic groups of the lipid moieties preferably have chain lengths of two to twenty-four carbon atoms and have zero to six double bonds. The aliphatic groups may be attached to the glycerol moiety by acyl, ether or vinyl ether bonds.

Synthetic Methods:

[0075] The lipid-nucleotide compounds described herein can be synthesized according to general methods applicable to all lipids and all antiviral nucleosides described below, as indicated in the flow diagram of Figure and demonstrated specifically in Examples 1 through 6.

[0076] Starting materials comprising fatty acids, alcohols, glycerides and phospholipids may be purchased from commercial suppliers (Avanti Polar Lipids, Inc., Pelham, Alabama 35124) or may be synthesized according to known methods. Antiviral nucleoside analogues are available from Aldrich, Milwaukee, Wisconsin or from Sigma, St. Louis, Missouri.

[0077] It is important that all traces of water be removed from the reactants in order for the coupling reactions to proceed. Therefore, the lipids are first either freeze-dried by solvent evaporation under vacuum, or in a vacuum oven over P_2O_5. The reactions are also carried out under an inert gas, such as, for example, argon.

[0078] The compounds described herein can be formed according to synthetic procedures which couple a phospholipid to a nucleoside analogue or which couple a phospholipid to a nucleoside analogue monophosphate or diphosphate, wherein the phosphate group is located on the ribose group of the nucleoside, at either the 3′ or preferably the 5′ location.

[0079] Lipids suitable for coupling to nucleosides, comprising primarily monoglycerides or diglycerides, ceramides and other lipid species described below, may be phosphorylated by treatment with appropriate agents, for example using phenyl phosphorodichloridate according to the procedure of Brown (32), by treatment with phosphorus oxychloride as in Example 6, or by other known phosphorylation procedures.

[0080] In the first type of synthesis, a phospholipid, such as, for example, a phosphatidic acid, is coupled to a selected nucleoside analogue at either the 3′ or 5′ hydroxyl by means of a coupling agent, such as, for example, 2, 4, 6-triisopropylbenzenesulfonyl chloride in the presence of a basic catalyst, for example, anhydrous pyridine, at room temperature. Other coupling agents, such as dicyclohexylcarbodiimide can be used.

[0081] Lipid derivatives may also be synthesized by coupling a phosphatidic acid to an antiviral nucleoside monophosphate through a pyrophosphate bond. In this procedure, the nucleoside monophosphate or diphosphate is converted to a derivative having a leaving group, for example, morpholine, attached to the terminal phosphate group, according to the procedure of Agranoff and Suomi (21) and as illustrated in Example 4, for preparing a derivative of AZT and Example 6, for a derivative of ddA. A coupling of the phosphatidic acid and the nucleoside phosphate morpholidate occurs on treatment of a dry mixture of the two reactants with a basic catalyst, such as anhydrous pyridine, at room temperature.

[0082] The reactions are followed using thin layer chromatography (TLC) and appropriate solvents. When the reaction, as determined by TLC is complete, the product is extracted with an organic solvent and purified by chromatography on a support suitable for lipid separation, for example, silicic acid.

[0083] The synthesis of products comprising adenine or cytidine having reactive amino groups may be facilitated by blocking those groups with acetate before the coupling reaction by treatment with acetic anhydride; after the chromatography of the final product, the amino groups are unblocked using ammonium hydroxide (Example 3).

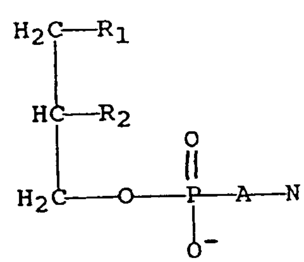
Lipid Derivatives:

[0084] The compounds have a lipid portion sufficient to be able to incorporate the material in a stable way into a liposomal bilayer or other macromolecular array.

[0085] Some preferred lipid derivatives of nucleoside analogues fall into four general classes:

1. Antiviral phosphatidylnucleosides:

[0086] The structure of these antiviral lipid compounds is shown below:



where N is a "chain terminating" dideoxynucleoside such as AZT, ddC, ddA, ddI, or another antiviral nucleoside such as acyclovir or ganciclovir, A is a chalcogen (O or S), and R_1 and R_2, which may be the same or different, are C_1 to C_{24} aliphatic groups, having from 0 to 6 sites of unsaturation, and having the structure

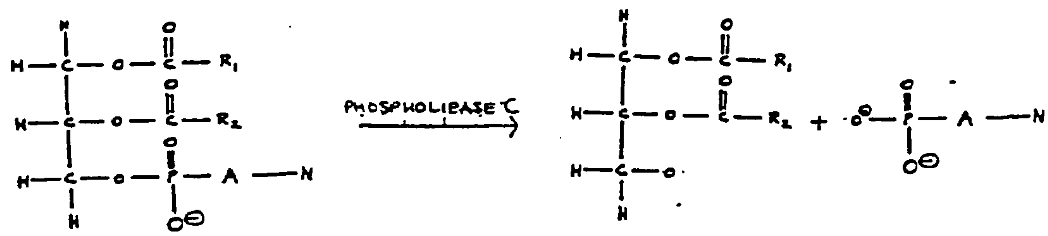
$$CH_3\text{-}(CH_2)_a\text{-}(CH{=}CH\text{-}CH_2)_b\text{-}(CH_2)_c\text{-}Y$$

wherein the sum of a and c is from 1 to 23; and b is 0 to 6; and wherein Y is C(O)O⁻, C-O⁻, C=C-O⁻, C(O)S-, C-S-, C=C-S-, forming acyl ester, ether or vinyl ether bonds, respectively, between the aliphatic groups and the glycerol moiety. These aliphatic groups in acyl ester linkage therefore comprise naturally occurring saturated fatty acids, such as lauric, myristic, palmitic, stearic, arachidic and lignoceric, and the naturally occurring unsaturated fatty acids palmitoleic, oleic,

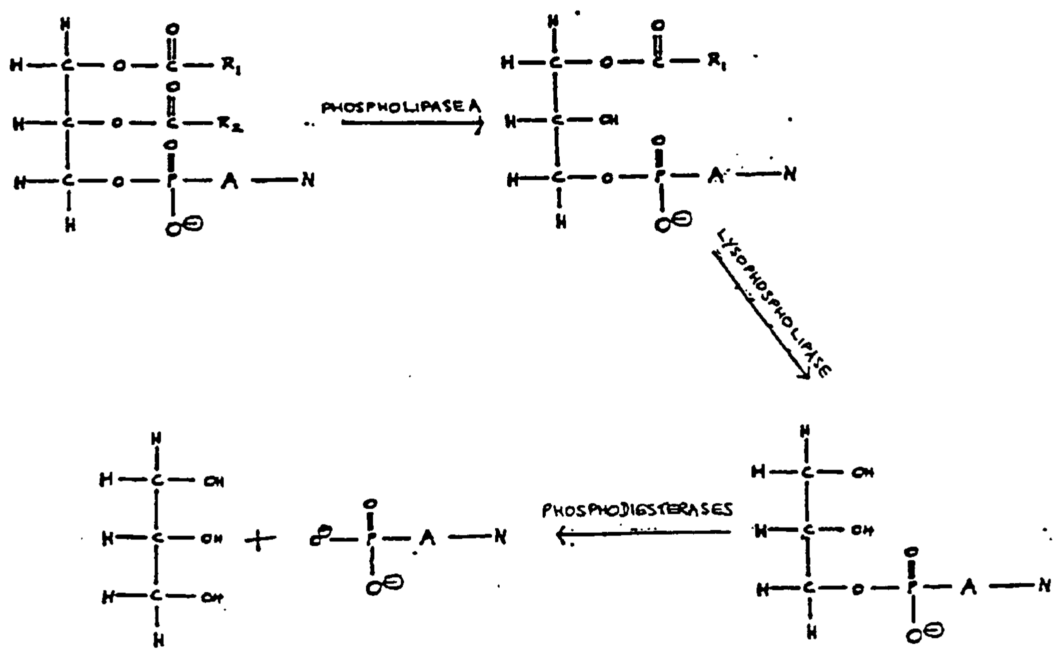
linoleic, linolenic and arachidonic. Preferred embodiments comprise a monoester or diester, or a 1-ether, 2-acyl ester phosphatidyl derivative. In other embodiments, the aliphatic groups can be branched chains of the same carbon number, and comprise primary or secondary alkanol or alkoxy groups, cyclopropane groups, and internal ether linkages.

[0087] This class of compounds may be prepared, for example, from the reaction of a diacylphosphatidic acid and an antiviral nucleoside analogue in pyridine as described for the preparation of 1,2 dimyristoylglycerophospho-5'-(3'-azido-3'-deoxy)thymidine in Example 1.

[0088] Upon liposomal uptake, the compounds are believed to undergo metabolism by the phospholipases present in the cell. For example, in the specific case of a diacylphosphatidyl derivative of a nucleoside, phospholipase C would act to give a diacylglycerol and the nucleoside monophosphate as shown below:

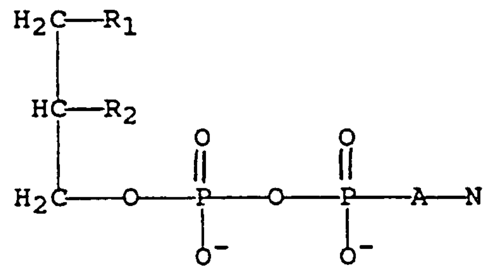


Alternatively, the same phosphatidylnucleoside may be hydrolyzed by phospholipase A and lysophospholipase followed by phosphodiesterase to give glycerol and nucleoside monophosphate by the sequence shown below:



2. <u>Antiviral nucleoside diphosphate diglycerides:</u>

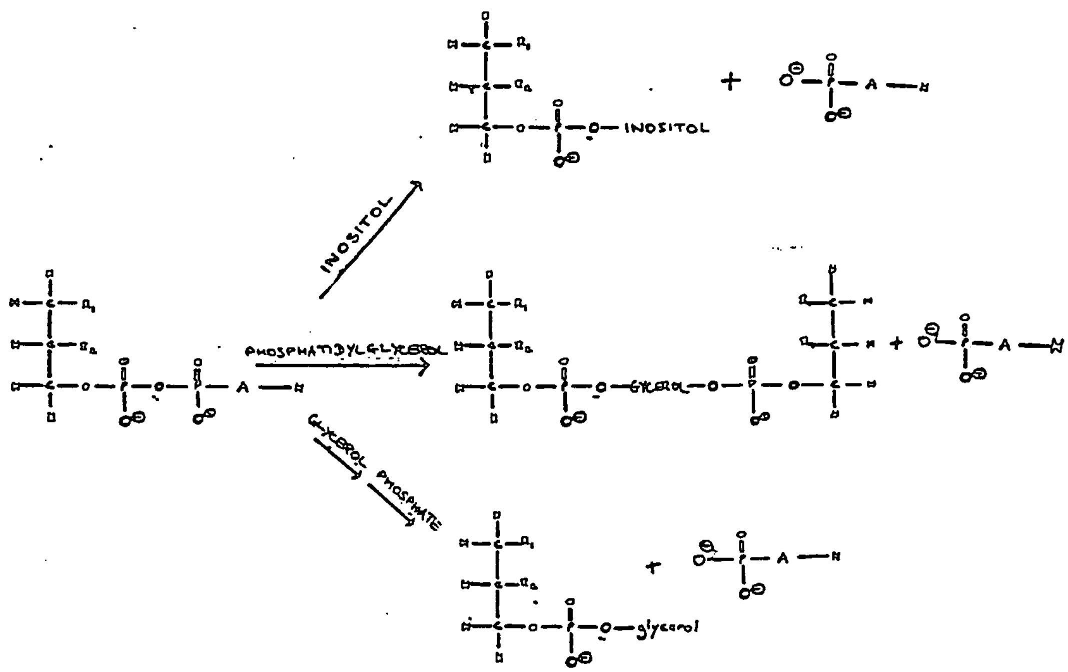
[0089] The chemical structure of this class of compounds is shown below:



where N, A and R_1 and R_2 are as described above.

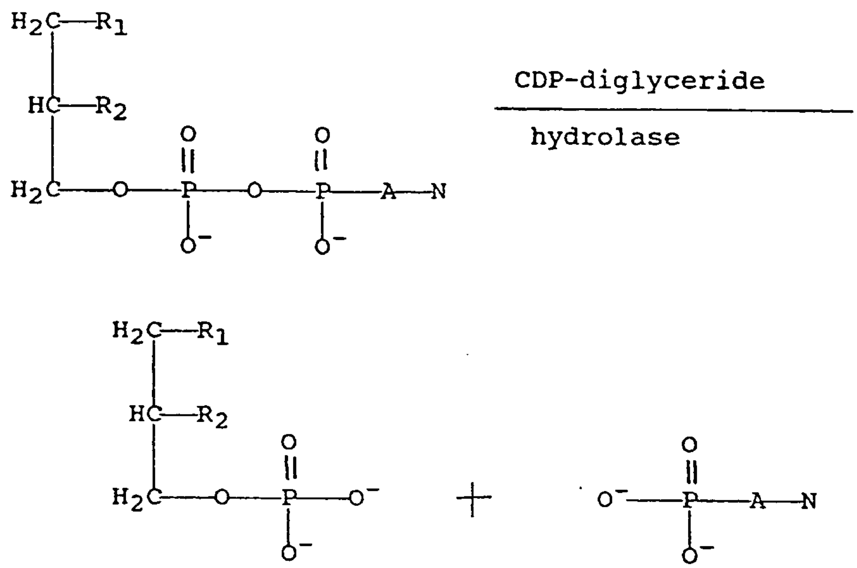
[0090] Nucleoside diphosphate diglycerides are known. The antiviral nucleoside diphosphate diglycerides may be prepared from phosphatidic acid and the antiviral nucleotide monophosphomorpholidates by the method of Agranoff and Suomi (21) as modified by Prottey and Hawthorne (22). This type of synthesis is presented in Example 4 for the synthesis of AZT 5'-diphosphate dipalmitoyl glycerol.

[0091] Upon liposomal delivery to cells, this class of compounds will take part in several types of reactions since it is an analogue of CDP-diglyceride, an important naturally-occurring intermediate in the biosynthesis of phosphatidylglycerol, cardiolipin and phosphatidylinositol as shown below:



[0092] All of these reactions generate nucleoside monophosphate and a new phospholipid. It is important to note that Poorthuis and Hostetler (23) showed previously that a variety of nucleosides could substitute for CDP-diglyceride in these reactions, including UDP-diglyceride ADP-diglyceride and GDP-diglyceride (23). Significantly, Ter Scheggett, et al. (24) synthesized deoxy CDP-diglyceride and found that it could also replace CDP-diglyceride in the mitochondrial synthesis of phosphatidylglycerol and cardiolipin, thereby suggesting the possibility of using these novel compounds to generate the antiviral nucleoside phosphates in the target cells.

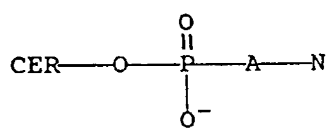
[0093] CDP-diglyceride hydrolase catalyzes another important metabolic conversion which gives rise to nucleoside monophosphate and phosphatidic acid, as shown below:



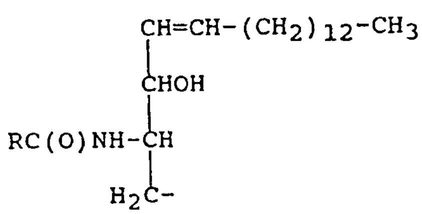
[0094] This pathway was first described in mammalian tissues by Rittenhouse, et al. (25). This enzyme, which is a pyrophosphatase, is expected to cleave dideoxynucleoside diphosphate diglyceride to the nucleoside monophosphate and phosphatidic acid, providing a second manner in which the nucleoside monophosphate can be formed in the target cells.

3. <u>Ceramide antiviral phosphonucleosides</u>:

[0095] Antiviral nucleoside phosphates can also be generated in cells after liposomal delivery of ceramide antiviral nucleoside phosphates having the general structure shown below:



where CER is an N-acylsphingosine having the structure:



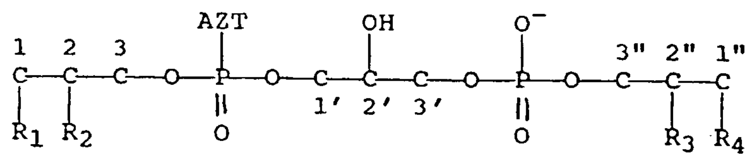
wherein R is as defined previously, or an equivalent lipid-substituted derivative of sphingosine, and N is a "chain terminating" antiretroviral nucleoside or antiviral nucleoside as previously defined. This class of compounds is useful in liposomal formulation and therapy of AIDS and other viral diseases because it can be acted upon by sphingomyelinase or phosphodiesterases in cells giving rise to nucleoside monophosphate. In addition to the compound shown above, ceramide diphosphate dideoxynucleosides can also be synthesized, which may be degraded by cellular pyrophosphatases to give nucleoside monophosphate and ceramide phosphate.

[0096] Ceramide antiviral nucleoside phosphates may be prepared in a method similar to the method for preparing

antiviral nucleoside diphosphate diglycerides, with appropriate changes to the starting materials.

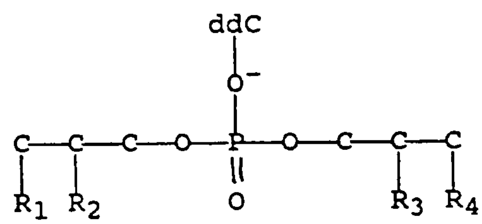
4. Other Lipid Derivatives of Antiviral Nucleosides

[0097] One approach to achieving even greater stability of lipid derivatives of nucleoside analogues within liposomes is by increasing lipid-lipid interaction between the lipid-nucleoside structure and the bilayer. Accordingly, in preferred embodiments, lipid derivatives of nucleoside analogues having up to four lipophilic groups may be synthesized. One class of these comprises diphosphatidylglycerol derivatives, having the general structure:



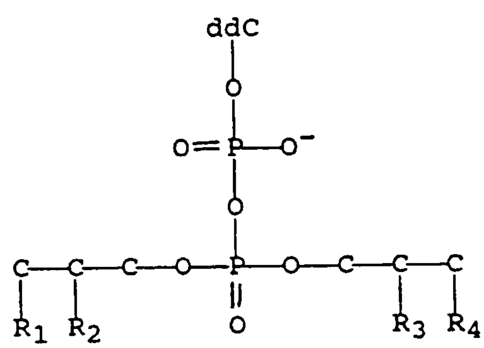
In this class, nucleosides are attached to one or both phosphates by a phosphodiester bond to the 5′-OH of the deoxyribose, ribose or dideoxyribose moiety of the antiviral nucleoside. In the case of acyclic nucleosides, such as acyclovir or ganciclovir, the link would be to the OH group equivalent to that of the ribose, deoxyribose or dideoxyribose 5′-position. There may be one or two nucleosides attached to each molecule. Nucleoside phosphates may also be attached by a pyrophosphate bond, as in Example 4.

[0098] Another class of derivatives having increased lipid components comprises bis(diacylglycero)phosphonucleotides, having the general structure:



R_{1-4} may be two, three or four aliphatic groups which are independently R as previously defined, said groups being in acyl ester, ether, or vinyl ether linkages. This compound may be made by the method of Example 3.

[0099] The diphosphate version of this compound, with the following structure:



may be made by coupling the nucleoside monophosphomorpholidate to the phosphoester residue of bis(diacylglycero)phosphate according to the procedure of Example 4. This compound will be metabolized to AZT-P in the cells by CDP-diglyceride hydrolase (a pyrophosphatase). These two types of compounds may provide superior metabolic and

physical properties.

[0100] Other suitable lipid derivatives of nucleosides may be synthesized using novel lipids. It is desirable, for example, to synthesize phospholipid derivatives of antiviral and antiretroviral nucleosides which will give rise to potent antiviral agents upon alternate paths of metabolism by the target cells which take up the lipid formulation. For derivatives made up of the following types of compounds, one might anticipate a cellular metabolism distinct from that of more conventional phospholipid derivatives, because these have a phosphate group which is removed from the usual lipid group by a nitrogen containing group. The structure of these lipids features a quaternary ammonium derivative. A preferred class comprises DL-2,3-diacyloxyethyl-dimethyl-betahydroxy ethyl ammonium derivatives.

[0101] In any of the lipids derivatives described in the preceding sections 1 through 4 above, the nucleoside may be any antiviral nucleoside; R_{1-2} (as well as R_{3-4} for the bis(diacylglycero) species) may be any saturated or unsaturated fatty acid having from 2 to 24 carbon atoms. Polyunsaturated, hydroxy, branched chain and cyclopropane fatty acids are also possible. The stereochemistry of the glycerol moieties can include sn-1 or sn-3 phosphoester bonds or racemic mixtures thereof. There may be 1 or 2, (as well as 3, or 4 for the bis(diacylglycero) species) acyl ester groups, or alkyl ether or vinyl ether groups, as required.

[0102] A variety of other phospholipids may be linked to nucleosides, including, but not limited to phosphatidylglycerol derivatives. In this case the nucleoside phosphate will be added by esterification to one or more of the hydroxyls of the alcohol. Other glycolipids may also serve as the ligand to which the phosphate group of the nucleotide is attached by means of esterification to a glycolipid hydroxyl group.

[0103] Furthermore, antiviral nucleosides may be linked to the phosphate-substituted carbohydrate moieties of phospholipids or glycolipids, either natural or synthetic.

[0104] Phospholipids having alkyl chains attached by ether or vinyl ether bonds may also be used to prepare nucleotide derivatives for use in the present invention. Suitable phospholipids for this purpose comprise naturally occurring acetal phosphatides, or plasmalogens, comprising a long chain fatty acid group present in an unsaturated vinyl ether linkage. Alternatively, analogs of 1-O-alkyl glycerol or 2-O-alkyl glycerol may be prepared synthetically, and linked to a selected nucleotide. Derivatives of glycero-3-phospho-5'-azidothymidine are preferred, and may be prepared by condensing AZT monophosphate with various analogs of 1-O-aliphatic derivatives of glycerol having an aliphatic group of 2 to 24 carbon chain length at the 1-position of glycerol. The 1-O-aliphatic group may consist of a saturated or unsaturated aliphatic group. The 1-O-aliphatic glycerol residue may be racemic or stereospecific. This compound may be acylated with fatty acid chlorides or anhydrides resulting in the synthesis of 1-O-alkyl, 2-acyl-glycero-3-phospho-5'azidothymidine.

Preparation of Liposomes comprising Lipid Derivatives of Antiviral Nucleosides

[0105] After synthesis, the lipid derivative of the nucleoside analogue is incorporated into liposomes, or other suitable carrier. The incorporation can be carried out according to well known liposome preparation procedures, such as sonication, extrusion, or microfluidization. Suitable conventional methods of liposome preparation include, but are not limited to, those disclosed by Bangham, et al. (4), Olson, et al. (26), Szoka and Papahadjapoulos (27), Mayhew, et al. (28), Kim, et al. (29), Mayer, et al. (30) and Fukunaga, et al. (31).

[0106] The liposomes can be made from the lipid derivatives of nucleoside analogues alone or in combination with any of the conventional synthetic or natural phospholipid liposome materials including phospholipids from natural sources such as egg, plant or animal sources such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, sphingomyelin, phosphatidylserine, or phosphatidylinositol. Synthetic phospholipids that may also be used, include, but are not limited to, dimyristoylphosphatidylcholine, dioleoylphosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidycholine, and the corresponding synthetic phosphatidylethanolamines and phosphatidylglycerols. Other additives such as cholesterol or other sterols, cholesterol hemisuccinate, glycolipids, cerebrosides, fatty acids, gangliosides, sphingolipids, 1,2-bis(oleoyloxy)-3-(trimethyl ammonio)propane (DOTAP), N-[1-(2,3-dioleoyl) propyl]-N,N,N-trimethylammonium (chloride) (DOTMA), D,L,-2,3-distearoyloxypropyl(dimethyl)-β-hydroxyethyl ammonium (acetate), glucopsychosine, or psychosine can also be added, as is conventionally known. The relative amounts of phospholipid and additives used in the liposomes may be varied if desired. The preferred ranges are from about 80 to 95 mole percent phospholipid and 5 to 20 mole percent psychosine or other additive, Cholesterol, cholesterol hemisuccinate, fatty acids or DOTAP may be used in amounts ranging from 0 to 50 mole percent. The amounts of antiviral nucleoside analogue incorporated into the lipid layer of liposomes can be varied with the concentration of their lipids ranging from about 0.01 to about 100 mole percent.

[0107] Using conventional methods to entrap active compound entraps approximately 20 to 50% of the material present in solution; thus, approximately 50 to 80% of the active compound is wasted. In contrast, where the nucleoside analogue is incorporated into the lipids, virtually all of the nucleoside analogue is incorporated into the liposome, and virtually none of the active compound is wasted.

[0108] The liposomes with the above formulations may be made still more specific for their intended targets with the

incorporation of monoclonal antibodies or other ligands specific for a target. For example, monoclonal antibodies to the CD4 (T4) receptor may be incorporated into the liposome by linkage to phosphatidylethanolamine (PE) incorporated into the liposome by the method of Leserman, et al. (19). As previously described, HIV will infect those cells bearing the CD4 (T4) receptor. Use of this CD4-targeted immunoliposome will, therefore, focus antiviral compound at sites which HIV might infect. Substituting another CD4 recognition protein will accomplish the same result. On the other hand, substituting monoclonal antibody to gp110 or gp41 (HIV viral coat proteins) will focus antiviral immunoliposomes at sites of currently active HIV infection and replication. Monoclonal antibodies to other viruses, such as Herpes simplex or cytomegalovirus will focus active compound at sites of infection of these viruses.

Therapeutic Uses of Lipid Derivatives

[0109] The liposome incorporated phosphorylated nucleoside analogue is administered to patients by any of the known procedures utilized for administering liposomes. The liposomes can be administered intravenously, intraperitoneally, intramuscularly, or subcutaneously as a buffered aqueous solution. Any pharmaceutically acceptable aqueous buffer or other vehicle may be utilized so long as it does not destroy the liposome structure or the activity of the lipid nucleoside analogue. One suitable aqueous buffer is 150 mM NaCl containing 5 mM sodium phosphate with a pH of about 7.4 or other physiological buffered salt solutions.

[0110] The dosage for a mammal, including a human, may vary depending upon the extent and severity of the infection and the activity of the administered compound. Dosage levels for nucleoside analogues are well established. Dosage levels of lipid derivatives of nucleoside analogues should be such that about 0.001 mg/kilogram to 1000 mg/kilogram is administered to the patient on a daily basis and more preferably from about 0.05 mg/kilogram to about 100 mg/kilogram.

[0111] The present invention utilizes the antiviral nucleoside derivatives noted above incorporated in liposomes in order to direct these compounds to macrophages, monocytes and any other cells which take up the liposomal composition. Ligands may also be incorporated to further focus the specificity of the liposomes.

[0112] The derivatives described have several unique and novel advantages over the water soluble dideoxynucleoside phosphates described in an earlier copending application.

[0113] First, they can be formulated more efficiently. Liposomes comprising lipid derivatives of nucleoside analogues have much higher ratios of drug to lipid because they are incorporated into the wall of the liposome instead of being located in the aqueous core compartment. Secondly, the liposomes containing the lipophilic dideoxynucleoside derivatives noted above do not leak during storage, providing improved product stability. Furthermore, these compositions may be lyophilized, stored dry at room temperature, and reconstituted for use, providing improved shelf life. They also permit efficient incorporation of antiviral compounds into liposomal formulations without significant waste of active compound.

[0114] They also provide therapeutic advantages. Stability of the liposomally incorporated agent causes a larger percentage of the administered antiviral nucleoside to reach the intended target, while the amount being taken up by cells in general is minimal, thereby decreasing the toxic side effects of the nucleosides. The toxic side effects of the nucleosides may be further reduced by targeting the liposomes in which they are contained to actual or potential sites of infection by incorporating ligands specifically binding thereto into the liposomes.

[0115] Finally, the compounds noted above have been constructed in a novel way so as to give rise to phosphorylated dideoxynucleosides or other antiviral nucleosides upon further cellular metabolism. This improves their antiretroviral (antiviral) effect in monocytes and macrophages or other cells which are known to be resistant to the effects of the free antiviral compounds. Further, the compounds pre-incubated with lymphoid cells provide complete protection from HIV infection for up to and exceeding 48 hours after the drug is removed, while the free nucleoside provides no protection 24 hours after removal. Finally, the lipid compounds are expected to be useful in treating HIV infections due to strains of virus which are resistant to free antiretroviral nucleoside analogues.

[0116] Lipid derivatives of antiviral agents have a prolonged antiviral effect as compared to the lipid-free agents; therefore they provide therapeutic advantages as medicaments even when not incorporated into liposomes. Non-liposomal lipid derivatives of antiviral nucleoside analogues may be applied to the skin or mucosa or into the interior of the body, for example orally, intratracheally or otherwise by the pulmonary route, enterally, rectally, nasally, vaginally, lingually, intravenously, intraarterially, intramuscularly, intraperitoneally, intradermally, or subcutaneously. The present pharmaceutical preparations can contain the active agent alone, or can contain further pharmaceutically valuable substances. They can further comprise a pharmaceutically acceptable carrier.

[0117] Pharmaceutical preparations containing lipid derivatives of antiviral nucleosides are produced by conventional dissolving and lyophilizing processes to contain from approximately 0.1% to 100%, preferably from approximately 1% to 50% of the active ingredient. They can be prepared as ointments, salves, tablets, capsules, powders or sprays, together with effective excipients, vehicles, diluents, fragrances or flavor to make palatable or pleasing to use.

[0118] Formulations for oral ingestion are in the form of tablets, capsules, pills, ampoules of powdered active agent,

or oily or aqueous suspensions or solutions. Tablets or other non-liquid oral compositions may contain acceptable excipients, known to the art for the manufacture of pharmaceutical compositions, comprising diluents, such as lactose or calcium carbonate; binding agents such as gelatin or starch; and one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring or preserving agents to provide a palatable preparation. Moreover, such oral preparations may be coated by known techniques to further delay disintegration and absorption in the intestinal tract.

[0119] Aqueous suspensions may contain the active ingredient in admixture with pharmacologically acceptable excipients, comprising suspending agents, such as methyl cellulose; and wetting agents, such as lecithin or long-chain fatty alcohols. The said aqueous suspensions may also contain preservatives, coloring agents, flavoring agents and sweetening agents in accordance with industry standards.

[0120] Preparations for topical and local application comprise aerosol sprays, lotions, gels and ointments in pharmaceutically appropriate vehicles which may comprise lower aliphatic alcohols, polyglycols such as glycerol, polyethylene glycol, esters of fatty acids, oils and fats, and silicones. The preparations may further comprise antioxidants, such as ascorbic acid or tocopherol, and preservatives, such as p-hydroxybenzoic acid esters.

[0121] Parenteral preparations comprise particularly sterile or sterilized products. Injectable compositions may be provided containing the active compound and any of the well known injectable carriers. These may contain salts for regulating the osmotic pressure.

[0122] The therapeutically effective amount of the lipid derivatives is determined by reference to the recommended dosages of the active antiviral nucleotide, bearing in mind that, in selecting the appropriate dosage in any specific case, consideration must be given to the patient's weight, general health, metabolism, age and other factors which influence response to the drug. The parenteral dosage will be appropriately an order of magnitude lower than the oral dose.

[0123] A more complete understanding of the invention can be obtained by referring to the following illustrative examples, which are not intended, however, to unduly limit the invention.

EXAMPLE 1

Synthesis of 1,2-Dimyristoylglycerophospho-5'-(3'-azido-3'-deoxy)thymidine, monosodium salt.

Preparation of dimyristoylphosphatidic acid (DMPA-H):

[0124] In a separatory funnel (500 ml), dimyristoylphosphatidic acid disodium salt (1 g., 1.57 mmol) was first dissolved in chloroform:methanol (2:1 by volume, 250 ml) and mixed well. Distilled water (50 ml) was added to the solution, and the pH was adjusted to 1 by adding concentrated hydrochloric acid. The solution was mixed well and the chloroform layer collected. The chloroform layer was back washed once with methanol:water (1:1 by volume, 80 ml) and evaporated under reduced pressure at 30°C to yield dimyristoylphosphatidic acid (DMPA-H) as a white foam. Cyclohexane (10 ml) was added and the solution lyophilized to dryness to obtain a white powder (850 mg) which was then stored at -20°C. A day before the coupling reaction, DMPA-H (250 mg, 0.42 mmol) was dissolved in cyclohexane (10 ml) in a round-bottom (50 ml) flask and the solvent evaporated under reduced pressure at room temperature. This process was repeated four more times and the DMPA-H further dried in the vacuum oven at room temperature overnight over P_20_5 and stored in a desiccator at -20°C.

Coupling reaction:

[0125] Under argon, to the 50 ml round-bottom flask containing dried DMPA-H (250 mg, 0.42 mmol), dried 3'-azido-3'-deoxythymidine (AZT), Sigma Chemical, St. Louis, Missouri, (85 mg, 0.31 mmol, dried over P_2O_5 under vacuum overnight), and 2,4,6-triisopropylbenzenesulfonyl chloride (315 mg, 1.04 mmol) was added, and anhydrous pyridine (2 ml) added via syringe to obtain a clear solution. The reaction mixture was stirred at room temperature for 18 hours. (The reaction was followed by thin layer chromatography). Water (1 ml) was added to the crude product to destroy excess catalyst and the solvent was evaporated under reduced pressure to yield a yellow gum which was then redissolved in a small volume of methanol:chloroform (1:9 by volume) and applied to a column of silica gel (45 g, Kieselgel 60, West Germany). The column was eluted with 8% methanol in chloroform. After a forerun (rejected), AZT was recovered, and then dimyristoylphosphatidyl-3'-azido-3'-deoxythymidine (DMpAZT) was obtained. The fractions containing the product were combined and the solvent was evaporated under reduced pressure. Cyclohexane (5 ml) was added to the residue and the mixture lyophilized to dryness under vacuum over P_20_5 to yield pure DMpAZT (270 mg, 0.29 mmol, 95%).

Conversion to monosodium salt:

[0126] To the dried DMpAZT redissolved in chloroform:methanol (2:1 by volume, 30 ml), distilled water (6 ml) was

added, mixed well, and the pH of the aqueous layer was adjusted to 1. The chloroform layer was collected and 10 ml of methanol:water (1:1,) was added and mixed well. The pH of the aqueous layer was adjusted to 6.8 with methanolic NaOH (0.1N N), mixed well, and the aqueous layer was maintained at pH 6.8. The combined chloroform, methanol and water mixture was evaporated under reduced pressure to yield dimyristoylphosphatidyl 3'-azido-3'-deoxythymidine monosodium salt. The residue was redissolved in chloroform:methanol (2:1 by volume, 2 ml) and acetone added to precipitate DMpAZT monosodium salt which was further dried from cyclohexane (5 ml) to yield a white powder (220 mg, 0.26 mmol, 78% yield based on AZT). The melting point was 230°C; Rf value on silica gel G thin layer plates was 0.32 (chloroform:methanol:water:ammonia 80:20:1:1), Rf 0.58 (chloroform:methanol:water:ammonia 70:30:3:2), Rf 0.31 (chloroform:methanol:water 65:25:4); UV absorption maximum 266nm (ε 10,800); Analysis Calculated for $C_{41}N_5O_{11}P_1H_{72}$. 1 H_2O: C,57.24; H,8.44; P,3.61; Found: C,56.80; H,8.83; P,3.52. MS, m/e 864.60 (M+)
Proton NMR: (CDCL3) δ 0.88 (6H, bt, J=6.9Hz, acyl CH3), 1.26 (40H, s, acyl CH2), 1.60 (4H, bs, β acyl CH2), 1.94 (3H, s, thymine CH3), 2.31 (4H, m, α acyl CH2), 2.39 (2H, m, ribose 2'H), 3.38 (2H, bd, J=12.6Hz, ribose 5'H), 3.78 (2H, m, sn-3 CH_2 glycerol), 4.00 (1H, dd, J1=12Hz, J2=6Hz, sn-1 CH_2 glycerol), 4.18 (1H, dd, J1=12Hz, J2=6Hz, sn-1 CH_2 glycerol), 4.07 (1H, m, ribose 3'H), 4.41 (1H, m, ribose 4'H), 5.24 (1H, m, sn-2 CH glycerol), 7.62 (1H, s, thymine 6H), 6.21 (1H, t, J=6Hz, ribose 1'H). The peak area ratio of phosphatidic acid to AZT is 1.

EXAMPLE 2

Synthesis of 1,2-Dimyristoylglycerophospho-5'-(3'deoxy)thymidine, monosodium salt.

[0127] 3'-deoxythymidine was obtained from Sigma Chemical, St. Louis, Missouri. The lipid derivative of this analogue was synthesized using the same method described above in Example 1. Melting Point 235°C, Rf on silica gel G 0.25 (chloroform/methanol/water/ammonia 80:20:1:1); 0.57 (chloroform:methanol:ammonia:water 70:30:3:2); 0.24 (chloroform:methanol:water 64:25:4); UV absorption maximum 269 nm (ε 8,400); Analysis: Calculated for $C_{41}N_2O_{11}P_1H_{72}Na_1 \cdot 1H_2O$: C,58.53; H,8.87; P, 3.69; Found: C,56.75; H,9.33; P,3.58. MS, m/e 823.00 (M+).
Proton NMR: (CDCL3) δ 0.91 (6H, bt, J=6.8Hz, acyl CH3), 1.23 (4H, bs, acyl CH_2), 1.26 (4H, bs, acyl CH2), 1.28 (32H, bs, acyl CH2), 1.62 (4H, m, β acyl CH2), 1.97 (3H, s, thymine CH3), 2.05 (2H, m, ribose 2'H), 2.35 (4H, m, α acyl CH2), 3.39 (2H, bs, ribose 5'H), 3.90 (2H, m, sn-1 CH_2 glycerol), 4.16 (1H, m, sn-1 CH_2 glycerol), 4.24 (1H, m, sn-1 CH_2 glycerol), 4.38 (1H, m, ribose 4'H), 5.23 (1H, m, sn-2 glycerol) 6.10 (1H, bt, ribose 1'H), 7.68 (1H, s, thymine 6H). The peak area ratio of phosphatidic acid to 2'3'-dideoxythymidine is 1.

EXAMPLE 3

Synthesis of, 1,2-Dimyristoylglycerophospho-5'-(2',3'-dideoxy)cytidine

Preparation of 4-acetal-2'3'-dideoxycytidine:

[0128] To a stirred, refluxing solution of 2'-3'-dideoxycytidine (DDC) (400 mg, 1.89 mmol) in anhydrous ethanol (35 ml, dried first with Lindy type 4x molecular sieve, and twice distilled over magnesium turnings) was added acetic anhydride (0.4 ml, 5.4 mmol). During the course of a 3 hour refluxing period, four more additional 0.4 ml portions of acetic anhydride were added at 30 minute intervals. The reaction was followed by thin layer chromatography (silica gel F254, Kodak Chromagram, developed with 10% methanol in chloroform). After the final addition, the solution was refluxed for 1 more hour. The reaction mixture was cooled and solvent was evaporated under diminished pressure. The residue was redissolved in 8% methanol in chloroform (5 ml) and chromatographed on a silica gel column (2.2 cm x 30 cm, Kieselgel 60, 70-230 mesh, EM Science, 45 g). The column was eluted with 8% methanol in chloroform to yield pure 4-acetyl-2'3'-dideoxycytidine (ddC-OAC) in 80% yield.

Coupling reaction:

[0129] A day before the coupling reaction, DMPA-H (prepared as before, 250 mg, 0.42 mmol) was dissolved in cyclohexane (10 ml) in a round-bottom flask (50 ml) and the solvent evaporated under reduced pressure at room temperature. This process was repeated four more times and DMPA-H further dried in a vacuum oven at room temperature overnight over P_2O_5. Under argon, to the 50 ml round-bottom flask containing dried DMPA-H was added dried (ddC-OAC) (85 mg, 0.33 mmol, dried over P_2O_5 under vacuum overnight), and 2,4,6-triisopropylbenzenesulfonyl chloride (315 mg, 1.04 mmol), and anhydrous pyridine (2 ml) via syringe to obtain a clear solution. The reaction mixture was stirred at room temperature for 18 hours. (The reaction was followed by thin layer chromatography). Water (1 ml) was added to the mixture to destroy excess catalyst. The solvent was evaporated under reduced pressure to yield a yellow gum which was redissolved in a small volume of methanol in chloroform (1:9 by volume) and applied to a column of sil-

ica gel (45 g, Kieselgel 60, EM Science). The column was topped with a small amount of sand (500 mg) to prevent the sample from floating during elution. The column was eluted with 8% methanol in chloroform (1.5L). After a forerun (rejected), then dimyristoylphosphatidyl-5′-(2′3′-dideoxy)cytidine (DMpddC) was obtained. The fractions containing the product were combined and the solvent was evaporated under reduced pressure. The residue was further dried with cyclohexane to yield pure DMpddC-OAC (210 mg, 0.21 mmol, in 70% yield). R_f 0.40 (silica gel GF, 20x20 cm, Analtech, chloroform:methanol:water: ammonia 80:20:1:1 by volume).

Deblocking with 9N NH40H:

[0130] DMpddC-OAC (40 mg, 0.04 mmol) was dissolved in chloroform:methanol (1:1, 2 ml), and 9N NH_4OH (10 drops) was added at once. The solution was stirred at room temperature for 15 minutes and was then quickly neutralized with glacial acetic acid to pH 7. The neutralized solution was evaporated to dryness overnight under reduced pressure to yield dimyristoylphosphatidyl 5′(2′3′-dideoxy)cytidine (DMpddC, 35 mg, 0.037 mmol). Melting point: DMpddC decomposed at 240°C. On thin layer chromatography on silica gel GF plates, the Rf values were: 0.11 (chloroform:methanol: water: ammonia 80:20:1:1); 0.38 (chloroform:methanol:ammonia:water 70:30:3:2) ; 0.15 (chloroform:methanol:water 65:25:4); UV absorption maximum 273 nm (ε 5,800).
NMR: (CDCL3) δ 0.86 (6H, bt, acyl CH3), 1.24 (40H, bs, acyl CH2), 1.57 (4H, m, β acyl CH2), 2.28 (4H, m, α acyl CH2), 3.36 (2H, m, ribose 5'H), 3.94 (2H, bs, sn-3 CH_2 glycerol), 4.19 (1H, m, sn-1 CH_2 glycerol), 4.29 (1H, m, sn-1 CH_2 glycerol), 4.40 (1H, bs, ribose 4'H), 5.19 (1H, m, sn-2 CH glycerol), 5.89 (1H, m, thymine 5-H), 7.44 (1H, bs, thymine NH3), 7.94 (1H, bs, thymine NH_2). The peak area ratio of phosphatidic acid to 2'3'-dideoxycytidine is 1.

EXAMPLE 4

Synthesis of (3'azido-3'-deoxy)thymidine-5'-diphosphate-sn-3-(1,2-dipalmitoyl)glycerol

Synthesis of AZT-monophosphate morpholidate:

[0131] This compound was synthesized following the method of Agranoff and Suomi (21). AZT-monophosphate was converted into the acidic form by passing a solution in water through a column of Dowex 50W (50x2-200, 100-200 mesh, Sigma Chemicals, St. Louis, MO). A solution of 117 mg AZT-monophosphate (0.3 millimoles) in 3 ml of water was transferred to a two neck round bottom flask. The 3 ml of t-butanol and 0.106 ml of freshly distilled morpholine (1.20 millimoles) were added and the mixture was placed in a oil bath at 90°C. Four equivalents of dicyclohexylcarbodlimide 249 mg, 1.20 millimole) in 4.5 ml of t-butanol were added dropwise. The reaction was monitored by thin layer chromatography on silica gel 60, F 254, plates (E. Merck, Darmstadt) with chloroform/methanol/acetic acid/water (50/25/3/7 by volume) as the developing solvent. The reaction was noted to be complete after 3 hours. The mixture was cooled and after addition of 4.5 ml of water was extracted four times with 15 ml of diethylether. The aqueous layer was evaporated to dryness and dried in vacuo over P_2O_5. The product was obtained (199 mg, 100% yield) and used for coupling to phosphatidic acid without further purification.

Coupling of AZT-monophosphate morpholidate to dipalmitoylphospatidic acid:

[0132] Dipalmitoylphosphatidic acid, disodium salt was converted to the free acid by extracting the material from chloroform by the method of Bligh and Dyer (34) using 0.1N HCl as the aqueous phase. The chloroform layer was evaporated to dryness in vacuo, the phosphatidic acid (196 mg, 0.3 millimoles) was redissolved in chloroform and transferred to the vessel containing the AZT-monophosphate morpholidate. After the chloroform was removed in vacuo using a rotary evaporator, the mixture was dried by addition and evaporation of benzene and finally dried in vacuo over P_20_5. The reaction was started by addition of 30 ml of anhydrous pyridine and the clear mixture was stirred at room temperature. The reaction was monitored with thin layer chromatography as noted above with chloroform/methanol ammonia/water (70/38/82 by volume) as developing solvent. The Rf of phosphatidic acid, AZT-monophosphate morpholidate and AZT-diphosphate dipalmitoylglycerol is 0.11, 0.50, and 0.30, respectively.
[0133] After 70 hours the pyridine was evaporated and the product was extracted into chloroform after addition of 15 ml of water, 30 ml of methanol, 22 ml chloroform and sufficient 1N formic acid to adjust the pH to 4.0. The combined chloroform layers after two extractions were evaporated to dryness, the residue was dissolved in chloroform/methanol/ammonia/water, 70/38/8/2, and the product was purified by silica gel column chromatography in this solvent applying an air pressure equivalent to one meter of water. Fractions not completely pure were further purified by HPLC on a reverse phase column (Vydac C18) using water/methanol (8/2 by volume) and methanol as eluents. Fractions containing the desired product were evaporated to dryness to give 132 mg. of a white solid (44% yield) which gave a single spot by thin layer chromatography with silica gel g plates developed with chloroform/methanol/ammonia/water,

70/38/8/2 (Rf 0.35) and chloroform/methanol/water, 65/35/4 (Rf 0.54).
500 MHz NMR ($CDCl_3$) δ 0.88 (3H, t, J=6.93 Hz, sn-2-acyl CH_3), 0.92 (3H, t, J=7.48 Hz, sn-1 acyl chain CH_3), 1.25 (48H, s, CH_2 acyl chains), 1.55 (4H, bs, β CH_2 acyl chains) 1.83 (3H, s, CH_3 thymine), 2.25 (2H, t, J=6.97 Hz, 2H, alpha CH_2 sn-2-acyl chain), 2.27 (2H, t, J=7.79 Hz, α CH_2 sn-1-acyl chain), 2.44 (4H, bs, 2′ and 5′ H ribose), 3.78 (1H, dd, J=1.68, 5.51 Hz, 3′H ribose), 3.95 (2H, bs, sn-3 CH_2 glycerol), 4.07 (1H, bs, He/H_a sn-1 CH_2 glycerol), 4.13 (1H, bs, 1H, sn-2 CH glycerol), 4.36 (1H, bs, H_a/H_e sn-1 CH_2 glycerol), 5.21 (1H, bs, sn-2 CH glycerol), 5.66 (1H, bs, 1′H ribose), 7.14 (1H, d, J=6.25 Hz, 6H thymine). The ratio of acyl chains: glycerol:ribose: thymine as deduced from appropriate resonances amounted to 2.12:0.93:0.98:1.00. IR (KBr, disk) showed 2105 (azido), 1745 (c=o ester) and 1705 (c=o thymine) as identifiable bands.

EXAMPLE 5

Synthesis of an antiviral nucleoside diacyl phosphate

[0134] Dihexadecyl phospho-5′-dideoxycytidine is synthesized according to the method described in Example 1, except that the reactants are dideoxycytidine and dihexadecyl hydrogen phosphate. The starting material dihexadecyl hydrogen phosphate is synthesized from hexadecan-1-ol and phenyl phosphorodichloridate as first reported by D. A. Brown, et al. (32).

EXAMPLE 6

Synthesis of Dideoxyadenosine diphosphate ceramide an antiviral phosphonucleoside

[0135] The method of Example 2 is repeated, except that dideoxyadenosine monophosphate morpholidate is substituted for the dideoxycytidine monophosphate morpholidate. Ceramide phosphoric acid is prepared by the action of phosphorus oxychloride on ceramide. Ceramide phosphoric acid is substituted for the dimyristoyl phosphatidic acid. Similar results are obtained.

EXAMPLE 7

Preparation of Liposomes containing Antiretroviral Liponucleotides

[0136] 6.42 micromoles of dioleoylphosphatidylcholine, 3.85 micromoles of cholesterol, 1.28 micromoles of dioleoylphosphatidylglycerol and 1.28 micromoles of dimyristoylphosphatidyl-azidothymidine were mixed in a sterile 2.0 ml glass vial and the solvent was removed in vacuo in a rotary evaporator. In some experiments, dimyristoylphosphatidylazidothymidine was replaced with either dimyristoylphosphatidyldideoxythymidine, dimyristoylphosphatidyldideoxycytidine or azidothymidine diphosphate dimyristoylglycerol; control liposomes were prepared by omitting the antiviral liponucleotide. The dried film was placed under high vacuum overnight at room temperature to remove traces of solvent. The lipid film was hydrated at 30°C with 0.3 ml of sterile 10 mM sodium acetate buffer (pH 5.0) containing isotonic dextrose and the ampule was sealed. The mixture was vortexed intermittently for 10 minutes followed by sonication using a Heat Systems Ultrasonics sonicator with a cup horn generator (431B) at output control setting #9 for 90 to 120 minutes at which time the sample is clarified. This sonicated preparation was diluted with sterile RPMI buffer and added to the tissue culture wells at the concentration indicated.

EXAMPLE 8

Coupling of monoclonal antibodies to CD4 to an antiviral lipid-containing liposome

[0137] Dimyristoylphosphatidyl-AZT produced by the method of Example 1, dimyristoylphosphatidylcholine, cholesterol and dimyristoylphosphatidylethanolamine in a molar ratio of 39:39:20:2. 200 mg of this lipid mixture was dried in vacuo using a rotary evaporator to form a thin film in a 100 ml round-bottom flask. 1 ml of sterile phosphate buffered saline was added and the mixture shaken gently at 20°C. for 20 minutes, followed by ten 30-second cycles of vortexing to form multilamellar liposomes. The suspension was subjected to 5 cycles of extrusion through two stacked Nucleopore polycarbonate filters having pore diameters of 200 nm to produce a homogeneous liposomal population. Other methods may be used such as sonication, reverse phase evaporation and use of a French press or Microfluidizer (Microfluidics, Newton, Massachusetts).1 to 2 mg of OKT4a monoclonal antibodies to CD4 antigen are thiolated by incubation with 0.08 mM N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP). Untreated SPDP is removed by gel filtration through Sephadex G25. The voiding DTP-protein is reduced with 0.05 M dithiothreitol in 0.1 M acetate buffered

saline at pH 4.5 for 20 minutes, producing reduced thiolated antibody.

[0138] Liposomes produced by the method of Example 8, representing 5 micromoles of phospholipid are incubated overnight at room temperature with 1 mg of thiolated antibody in 0.20 ml of isotonic MES/HEPES buffer, pH 6.7. The resulting immunoliposomes are purified by the discontinuous metrizimide gradient method of Heath et al. (33) and sterilized by passage through 200 nm filters.

EXAMPLE 9

Inhibition of HIV Replication in Tissue Culture Cells by Lipid Nucleoside Conjugates

A. METHODS

Viral infection of Human T-cells:

[0139] The human T lymphoblastoid cell line, CCRG-CEM (hereafter referred to as CEM), was grown in RPMI 1640 medium containing 100 U/ml penicillin G, 100 ug/ml streptomycin, 2 mM glutamine and 10% fetal bovine serum (Hyclone Laboratories, Logan, Utah). Cells were infected with the LAV-1 strain (L. Montagnier, Paris, France) at a multiplicity of infection of one tissue culture 50% infectious dose ($TCID_{50}$)/cell for 60 minutes at 37°C in medium containing 1% polybrene. CEM cells were infected in suspension at 6 X 10^4 cells/ml, washed three times by centrifugation and resuspension and then distributed in 96-well plates at 6 X 10^4 cells/well before addition of medium containing the liposomal antiretroviral liponucleotide drugs.

Antiviral Activity as determined by HIV p24 Assay:

[0140] Antiviral activity was assayed after 3 days by the inhibition of the production of HIV p24 (gag) antigen in the cell free culture medium of the infected cells exposed to different concentrations of drug; p24 antigen was measured by ELISA (Abbott Laboratories, Chicago, IL) according to the manufacturer's instructions. The data are the average of two determinations and are expressed as percentage of a control incubated in the absence of drugs.

B. Experiment H533-1: Figure 1

[0141] Liposomes containing 10 mole percent of either dimyristoylphosphatidylazidothymidine (abbreviation: DMpAZT) (LN1), dimyristoylphosphatidyldideoxythymidine (abbreviation: DMpddT) (LN2) or azidothymidine diphosphate dimyristoylglycerol (abbreviation: DMG-dp-AZT) (LN4) in the indicated concentrations were tested for their ability to inhibit HIV replication in CEM (wild type) cells in vitro. All three of these antiretroviral liponucleotides inhibited HIV p24 production; the amounts of drug required to reduce virus production by 50% (E.D. 50) were as follows:

DMpAZT (LN1)	2 uM
DMpddT (LN2)	30 uM
DMG-dp-AZT (LN4)	8 uM

[0142] This demonstrates that the lipid derivatives of antiretroviral nucleotides can enter CEM cells and be converted to active nucleoside as predicted. The control liposomes (CONT) which did not contain any antiretroviral nucleotide had no effect on p24 production by CEM cells.

C. Experiment H747-1a: Figure 2

[0143] Dimyristoylphosphatidylazidothymidine (DMpAZT) in liposomes (LN1) was compared with free azidothymidine (abbreviation: AZT) (N1). At low concentrations below 0.1 uM free AZT was more effective than the liponucleotide. At concentrations ranging from 2 to 170 uM the DMpAZT liposomes were more effective than the free AZT. Control liposomes (CONT) containing only inactive lipids as noted in methods were ineffective in reducing p24.

D. Experiment H747-1b: Figure 3

[0144] Dideoxythymidine (abbreviation: ddT) (N2) is a weak inhibitor of HIV p24 production. Surprisingly, DMpddT (LN2) is somewhat more effective than the free nucleoside. As can be seen in the chart, slightly more free ddT is required to reduce p24 production than with DMpddT. Control liposomes (CONT) at a matched total phospholipid concentration are without effect.

E. Experiment H637-1b: Figure 4

[0145] In this experiment, CEM cells were replaced with mutant cells (provided by Dr. Dennis Carson, Scripps Clinic, San Diego, CA) which lack the thymidine kinase enzyme (CEM tk-). These cells are unable to phosphorylate thymidine derivatives and AZT is therefore inactive since it cannot be converted to the active triphosphate derivative which is needed to inhibit HIV p24 replication. As shown in the chart, AZT (N1) is completely without effect on p24 production over a wide range of concentrations (0.2 to 100 uM). However, both DMpAZT (LN1) and DMpddT (LN2) were capable of reducing p24 production, proving that these compounds are metabolized in the cell to the nucleoside-monophosphate which can be further activated to the triphosphate by other cellular enzymes. This data provides proof of the principles outlined in the patent which predict direct metabolism to the nucleoside monophosphate.

F. Experiment H805-1: Figure 5

[0146] In this experiment dimyristoylphosphatidyldideoxycytidine (abbreviation: DMpddC) (LN3) and dimyristoylphosphatidyldideoxythymidine (DMpddT) (LN2) were compared with the effects of free AZT (N2) and dideoxycytidine (abbreviation: ddC) (N3) in CEM (wild type) cells in vitro. DMpddC was the most potent liponucleotide (ED_{50} 1.1 uM) and DMpddT was less active as noted before (ED_{50} 20 uM). Free liposomes without added antiretroviral nucleotide (CONT) were inactive.

G. Experiment I276:

[0147] In this experiment, antiviral protection provided by preincubation with dimyristoylphosphatidylazidothymidine (DMpAZT) (LN1) in liposomes prepared as noted above was compared with that of free azidothymidine (AZT) (N1). CEM (wild type) cells were preincubated for 3 days under standard conditions in RPMI media containing 7.14 µM of either free AZT (N1) or DMpAZT (LN1). The cells were then washed twice with PBS, and fresh RPMI media added. Each group of cells was then divided into three batches. One batch was immediately infected with HIV, as noted above; after washing away unattached HIV, the sample was allowed to incubate in media alone for 3 days. Two other batches were allowed to incubate in media alone for either 24 or 48 hours to allow any intracellular antiviral agent present to become depleted. Then they were infected with HIV, the cells washed free of virus, and fresh RPMI media added. After 3 days of further incubation, the supernates of all batches were tested for the presence of p24 protein.

Control Cells: CEM cells were subjected to HIV infection without preincubation; drug was added following HIV infection as indicated, and the cells were incubated for 3 days.
Preincubated Cells: CEM cells were preincubated for 3 days with media containing AZT (N1) or DMpAZT (LN1); after 3 days the cells were washed, subjected to HIV infection followed by addition of media without drugs. After a further incubation for 3 days, p24 was measured.

RESULTS:

[0148]

CEM Controls: No Preincubation	p24: ng/ml after 3 days incubation
HIV infection only	204; 207
HIV + 7.14 µM AZT (N1)	64; 69
HIV + 7.14 µM DMpAZT (LN1)	16; 16

CEM Preincubated Cells	Pre-Infection Interval without Drug	p24: ng/ml after 3 days incubation
7.14 μM AZT (N1)	24 h	404; 433
	48 h	271; 245
7.14 μM DMpAZT(LN1)	24 h	6; 7
	48 h	4; 15

[0149] After a 3 day preincubation, followed by 48 hours of incubation in normal media after removal of the drugs, DMpAZT provided complete protection from HIV replication as assessed by the reduced p24 production. However, AZT preincubation failed to protect the cells from HIV infection 24 and 48 hours after removal of the drug.

EXAMPLE 10

HIV Paired Isolate:Antiviral Sensitivity I.C.$_{50}$, μM; HT4-6C Plaque Reduction Assay

[0150] Methods: HT4-6C cells (CD4+ HeLa cells) were obtained from Dr. Bruce Chesebro, Rocky Mountain National Laboratories, Hamilton, MT), and infected with HIV isolates as noted in Example 9. After a 3 day incubation, the cells were washed, fixed and stained with crystal violet and plaques were counted. Clinical samples of HIV were isolated before AZT therapy (Pre) and 6 to 12 months after AZT treatment (Post). (Richman, D.D., Larder, B., and Darby, G., Manuscript submitted for publication, 1989). Using the HT4-6C plaque reduction assay, the sensitivity of the paired clinical isolates was determined using either AZT, DMpAZT or DMpddT.

ISOLATE	AZT	DMpAZT	DMpddT
A012			
Pre (G762-3)	0.01	0.53	4.2
Post (G691-2	2	0.37	6.6
A018			
Pre (H112-2)	0.01	0.47	7.4
Post (G910-6	4	0.59	6.3
A036			
Pre (G174-6c)	0.007	0.42	7.4
Post (G704-2)	5.6	0.59	4.2
PCP022			
Pre (H112-5)	0.03	0.47	4.2
Post (G780-1)	5.6	1.05	6.6
PCP026			
Pre (H112-6)	0.01	0.33	6.6
Post (G890-1)	2.8	0.74	2.6
Abbreviations: pAZT, phosphatidylazidothymidine; pddT, phosphatidyldideoxythymidine			

[0151] Post AZT treatment, all 5 isolates showed marked decreases in sensitivity to AZT. This was not observed to occur with DMpAZT and DMpddT indicating that the post-AZT isolates retain their usual level of sensitivity to the antiretroviral nucleoside administered in the form of novel phospholipid derivatives.

EXAMPLE 11

Synthesis of DimyristoylPhosphatidylacyclovir and Efficacy in Herpes Simplex Virus-Infected WI-38 Cells

[0152] Dimyristoylphosphatidic acid (disodium salt) was obtained from Avanti Polar Lipids, Birmingham, AL, and converted to the free acid (DMA-H) as described above in Example 1. Acycloguanosine (acyclovir, Zovirax^R) was obtained from Sigma Chemical Co., St. Louis, MO and 73 mg (0.32 mmol) was dried overnight over phosphorus pentoxide in a vacuum oven. 250 mg of DMPA-H (0.42 mmol) was added to a 50 ml round bottom flask and dried overnight over phosphorus pentoxide in a vacuum oven. Under dry argon, 73 mg of acycloguanosine, 315 mg (1.04 mmol) of triisopropylbenzenesulfonly chloride (Aldrich, Milwaukee, WI) and 2 ml of dry pyridine (Aldrich, Milwaukee, WI) were added to the round bottom flask. The reaction mixture was stirred at room temperature for 18 hours followed by the addition of 1 ml of distilled water.

[0153] The solvent was evaporated in vacuo to yield a yellow gum which was redissolved ina small volume of chloroform/methanol (9/1) and applied to a column of silica gel (45 gm: Kieselgel 60, EM Science, Cherry Hill, JN). The column was eluted with 8% methanol in chloroform (500 ml), 10% methanol in chloroform (250 ml) followed by 15% methanol in chloroform (1.5 L). After a 1.5 liter forerun rejected), dimyristoylphosphatidylacycloguanosine (DMpACV) was obtained. Three fractions were collected and analyzed: fraction 1 (200 ml, 130 mg DMpACV) continued pure DMpACV; fraction 2 (200 ml, 150 mg) and fraction 3 (200 ml, 50 mg) contained DMpACV and small amounts of starting material as impurities. Fraction 1 was concentrated in vacuo and to the residue was added 5 ml of cyclohexane; the solution was frozen and lyophilized to dryness under phosphorus pentoxide to yield pure dimyristoylphosphatidylacycloguanosine (80 mg, 0.1 mmol).

[0154] The purified compound gave a single spot with an Rf of 0.29 when applied to K6G silica gel plates (Whatman International, Maidstone, England) developed with chloroform/methanol/water/ammonia (70/30/1 by volume) . The UV absorption was maximal at 256 nm (extinction coefficient = 8.4 x 10^3 in $CHCl_3$). The percentage phosphorus was 3.30% (theoretical 3.89%) and the melting point was 245°C. On HPLC analysis, dimyristoylphosphatidylacycloguanosine gave a single peak with a retention time of 11 minutes (Spheri-5; Brownlee Labs, Applied Biosystems, Santa Clara, CA) when eluted with a mobile phase of 1-propanol/0.25 mM potassium phosphate/hexane/ethanol/acetic acid (245/179/31/50/0.5 by volume) at a flow rate of 0.5 ml/min.

Cell Cultures

[0155] Wi-38 cells were obtained from American Type Culture Collection (Rockville, Maryland 20852) and grown in Dulbecco's minimum essential medium (DMEM) with 10% fetal calf serum (FCS). The cells were grown in 250 cm square bottles until reaching confluence.

Virus

[0156] Herpes simplex virus (HSV) type 1 (HSV-1) and type 2 (HSV-2) were obtained from the American Type Culture Collection. Both virus stocks were prepared in Wi-38 cells; extensive cytopathic effects (CPD) were observed when the stock virus was harvested by a single freezing and thawing and the cell debris was clarified by low speed centrifugation (2000 rpm). Supernatant fluids containing the virus were aliquoted into small vials and stored at80C. Both HSV-1 and HSV-2 stocks were titered in Wi-38 cells before use in the experiments.

Herpes Simples Virus Plaque Reduction Assay

[0157] The plaque reduction assay was used to measure the antiviral effect of dimyristoyl-phosphatidylacyclovir or free ACV. Wi-38 cells were trypsinized with 0.25% trypsin for 5 min. The cells were harvested and centrifuged to remove residual trypsin and the cell pellet was resuspended in DMEM with 10% FCS. The Wi-38 cells were plated in a 96 well plate (5x10 cells/well) for one hour. The infected cells were then treated with dimyristoyl-phosphatidylacyclovir or ACV. The antiviral agents were prepared in stock solutions which were then diluted two-fold with 2% FBS in DMEM containing 0.5% methylcellulose. 100µl of each diluted antiviral agent was added into each well of HSV infected cells.

[0158] The control and drug-treated cell cultures were incubated in a 37°C incubator with 5% carbon dioxide for 24 hours. When HSV-infected cells (control without antiviral agent) showed readable number of plaques, the entire plate was fixed with methanol and stained with 1% crystal violet for 10 min. The dye was rinsed off with tap water and the

plate was dried and plaques were counted. The antiviral effect of ACV or dimerystoylphosphatidylacyclovir was determined by measurement of plaque reduction as shown in the example below.

RESULTS: EFFECT OF ACYCLOVIR AND DIMYRISTOYL-PHOSPHATIDYLACYCLOVIR ON PLAQUE FORMATION BY HSV-1 IN WI-38 CELLS				
Acyclovir conc	1	2	mean	% no Drug
10 uM	0	0	0	0
5	0	0	0	0
2.5	0	0	0	0
1.25	4	3	3.5	13
0.625	8	6	7	26
0.31	17	19	20	65
0.155	18	22	20	73
0	20;30	30;30	27.5	100
DMPhosphatidylACV	1	2	mean	% no Drug
214 uM	toxic	toxic	-	-
107	0	0	0	0
54	0	0	0	0
27	2	3	2.5	9
13.4	4	6	5	18
6.7	6	9	7.5	27
3.3	10	12	11	40
1.67	17	20	18.5	67
0.84	24	26	25	91
0	20;30	30;30	27.5	100

[0159] The data shown above indicate that dimyristoyl-phosphatidylacyclovir is effective in HSV-1 infected Wi-38 cells, the concentration which produces 50% inhibition is 2 uM versus 0.4 uM for acyclovir. Similar results were obtained with HSV-2 in infected Wi-38 cells.

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[0160]

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Claims

1. Use in the preparation of a composition for the treatment of a viral or retroviral infection in a mammal of a liponucleotide compound comprising

   a conjugate of an antiviral nucleoside analogue which does not occur naturally in the mammal to be treated and which is characterized by the ability to exert antiviral activity on DNA or RNA viruses, and a lipid moiety linked to the 5' position of the nucleoside analogue
   wherein the conjugate has the formula

$$(L)_m\text{-}(W)_n\text{-}A\text{-}Q\text{-}Z$$

   wherein

   Z is the base portion of a nucleoside analogue;
   Q is a pentose residue or an acyclic fragment thereof or a carbocyclic analogue;
   A is O or S;
   W is phosphate;
   n is 1 to 3;

   and L is a lipid moiety, wherein m = 1 to 5; wherein L is linked directly to W, and wherein L is selected from

$$\begin{array}{c} H_2\text{-}C\text{ - }R_1 \\ | \\ HC\text{ - }R_2 \\ | \\ H_2\text{-}C\text{ -} \end{array} \quad ; \quad \begin{array}{c} CH{=}CH\text{-}(CH_2)_{12}\text{-}CH_3 \\ | \\ HCOH \\ | \\ RC(O)NH\text{-}CH \\ | \\ H_2C\text{ -} \end{array} \quad ;\text{ and } \quad \begin{array}{c} H_2C\text{ - }R_1 \\ | \\ HC\text{ - }R_2 \\ | \\ (CH_3)_2\text{-}\overset{}{\underset{\oplus}{N}}\text{-}(CH_2)_2\text{-} \end{array}$$

wherein R, R_1 and R_2 independently have from 0 to 6 sites of unsaturation, and have the structure

$$CH_3\text{-}(CH_2)_a\text{-}(CH{=}CH\text{-}CH_2)_b\text{-}(CH_2)_c\text{-}Y$$

wherein the sum of a and c is from 1 to 23; and b is 0 to 6; and wherein Y is -C(O)O-, -C-O-, -C=C-O-, -C(O)S-, -C-S-, or -C=C-S-.

2. Use according to claim 1, wherein the non-naturally occurring nucleoside component is an analogue with modification of a naturally occurring base or pentose by virtue of substitution, deletion or replacement.

3. Use according to claims 1 or 2, wherein the pentose residue is a 2',3'-deoxy, 2',3'-dideoxy-2',3'-didehydro, azido or halo derivative of ribose, or an acyclic hydroxylated fragment of ribose.

4. Use according to claims 1 to 3, wherein the group -A-Q-Z is selected from the group consisting of

2',3'-dideoxycytidine;
2',3'-dideoxythymidine;
2',3'-dideoxyguanosine;
2',3'-dideoxyadenosine;
2',3'-dideoxyinosine;
2,6-diaminopurine-2',3'-dideoxyriboside;
2',3'-dideoxy-2',3'-didehydrothymidine;
2',3'-dideoxy-2',3'-didehydrocytidine carbocyclic;
2',3'-dideoxy-2',3'-didehydroguanosine;
3'-azido-3'-deoxythymidine;
3'-azido-3'-deoxyguanosine;
2,6-diaminopurine-3'-azido-2',3'-dideoxyriboside
3'-fluoro-3'-deoxythymidine;
3'-fluoro-2',3'-dideoxyguanosine;
2',3'-dideoxy-2'-fluoro-ara-adenosine;
2,6-diaminopurine-3'-fluoro-2',3'-dideoxyriboside
9-(4'-hydroxy-1',2'-butadienyl)adenine;
3-(4'-hydroxy-1',2'-butadienyl)cytosine;
9-(2-phosphonylmethoxyethyl)adenine;
3-phosphonomethoxyethyl-2,6-diaminopurine;
acyclovir or
ganciclovir.

5. Use according to any one of the preceding claims, wherein the nucleotide compound is present in a liposome or is capable of forming a liposome by itself.

6. An antiviral or antiretroviral liponucleotide of formula

$$(L)_m\text{-}(W)_n\text{-}A\text{-}Q\text{-}Z$$

wherein

Z is the base portion of a nucleoside analogue;
Q is a pentose residue or an acyclic fragment thereof or a carbocyclic analogue;
A is O or S;
W is phosphate;
n is 1 to 3;
L is a lipid moiety;
m is 1

wherein the lipid moiety is selected from

$$\begin{array}{c} H_2\text{-}C - R_1 \\ | \\ HC - R_2 \\ | \\ H_2\text{-}C - \end{array} \quad ; \quad \begin{array}{c} CH{=}CH\text{-}(CH_2)_{12}\text{-}CH_3 \\ | \\ HCOH \\ | \\ RC(O)NH\text{-}CH \\ | \\ H_2C - \end{array} \quad ; \text{ and } \quad \begin{array}{c} H_2C - R_1 \\ | \\ HC - R_2 \\ | \\ (CH_3)_2\text{-}\overset{}{\underset{\oplus}{N}}\text{-}(CH_2)_2\text{-} \end{array}$$

wherein R, R_1 and R_2 independently have from 0 to 6 sites of unsaturation, and have the structure

$$CH_3\text{-}(CH_2)_a\text{-}(CH{=}CH\text{-}CH_2)_b\text{-}(CH_2)_c\text{-}Y$$

wherein the sum of a and c is from 1 to 23; and b is 0 to 6; and wherein Y is -C(O)O-, -C-O-, -C=C-O-, -C(O)S-, -C-S-, or -C=C-S-,

- with the proviso that the compound is in the form of a liposome, when the pentose residue is arabinofuranose and the base portion is cytosine or adenine,
  with the proviso that when the lipid is a 1,2-diradylglycerol, and the linkage is 3-monophosphate, the nucleoside analogue is not 5-fluoro-uridine, 5-fluorocytidine, bredinin, tubercidin, arabinosyl-cytosine, arabinosyl-5-fluorocytosine, arabinosyl-5-fluorocytosine, arabinosyl-adenine, arabinosyl-thymine, 5-fluoro-2'-deoxyribo-uridine, and neplanocin A;
  with the proviso that when the lipid moiety is 1,2-diradyl or 1,3-diradylglycerol, and the linkage is monophosphate, the nucleoside is not cytidine, uridine, arabinosylcytosine, adenosine, guanosine, cyclocytidine, deoxyadenosine, deoxycytidine, deoxyguanosine, deoxythymidine, deoxyuridine, or inosine;
  with the proviso that when the lipid moiety is 1,2-di-O-acylglycerol, and the linkage is 3-diphosphate, the nucleoside is not cytidine, arabinosylcytosine, arabinosyladenine, 7-deazaadenosine; provided that when the lipid moiety is 1,2-di-O-alkylglycerol, and the linkage is 3-diphosphate, the nucleoside is not arabinosylcytosine; provided that when the lipid moiety is 1,2-di-O-acylglycerol, and the linkage is monophosphate, the nucleoside is not arabinosylcytosine;
  and with the proviso that when the lipid moiety is 1-O-alkyl-2-O-acylglycerol, the sugar is ribose, 2'-deoxyribose, arabinose or 2',2'-dihydroxyribose, and the linkage is diphosphate, the nucleoside does not contain a base which is adenine, cytosine, 5-fluorouracil, 5-azacytosine, 6-mercaptopurine or 7-deazaadenine.

7. A compound according to claim 6, wherein the group A-Q-Z is selected from the nucleosides comprising

2',3'-dideoxycytidine;
2',3'-dideoxythymidine;
2',3'-dideoxyguanosine;
2',3'-dideoxyadenosine;
2',3'-dideoxyinosine;
2,6-diaminopurine-2',3'-dideoxyriboside;
2',3'-dideoxy-2',3'-didehydrothymidine;
2',3'-dideoxy-2',3'-didehydrocytidine carbocyclic;
2',3'-dideoxy-2',3'-didehydroguanosine;
3'-azido-3'-deoxythymidine;

3'-azido-3'-deoxyguanosine;
2,6-diaminopurine-3'-azido-2',3'-dideoxyriboside
3'-fluoro-3'-deoxythymidine;
3'-fluoro-2',3'-dideoxyguanosine;
2',3'-dideoxy-2'-fluoro-ara-adenosine;
2,6-diaminopurine-3'-fluoro-2',3'-dideoxyriboside
9-(4'-hydroxy-1',2'-butadienyl)adenine;
3-(4'-hydroxy-1',2'-butadienyl)cytosine;
9-(2-phosphonylmethoxyethyl)adenine;
3-phosphonomethoxyethyl-2,6-diaminopurine;
acyclovir or
ganciclovir.

8. Compounds of claim 6 selected from the group consisting of

phosphatidyl(3'-azido-3'-deoxy)thymidine (pAZT);
phosphatidyl(2',3'-dideoxy)cytidine (pddC);
phosphatidyl(2',3'-dideoxy)thymidine (pddT);
(3'-azido-3'-deoxy)thymidine diphosphate diglyceride (AZTdpdg);
phosphatidylacyclovir (pACV);
1-O-stearylglycero-rac-3-phospho-5'-(3'azido,3'-deoxy)thymidine.

9. A liposome formed at least in part from a liponucleotide compound comprising

a conjugate of an antiviral nucleoside analogue which does not occur naturally in the mammal to be treated and which is characterized by the ability to exert antiviral activity on DNA or RNA viruses, and a lipid moiety linked to the 5' position of the nucleoside analogue
wherein the conjugate has the formula

$$(L)_m\text{-}(W)_n\text{-A-Q-Z}$$

wherein

Z is the base portion of a nucleoside analogue;
Q is a pentose residue or an acyclic fragment thereof or a carbocyclic analogue;
A is O or S;
W is phosphate;
n is 1 to 3;

and L is a lipid moiety, wherein m = 1 to 5; wherein L is linked directly to a W, and wherein L is selected from

$$\begin{array}{c} H_2\text{-C - }R_1 \\ | \\ HC\text{ - }R_2 \\ | \\ H_2\text{-C -} \end{array} ;\quad \begin{array}{c} CH{=}CH\text{-}(CH_2)_{12}\text{-}CH_3 \\ | \\ HCOH \\ | \\ RC(O)NH\text{-}CH \\ | \\ H_2C\text{ -} \end{array} ;\text{ and }\quad \begin{array}{c} H_2C\text{ - }R_1 \\ | \\ HC\text{ - }R_2 \\ | \\ (CH_3)_2\text{-}\overset{+}{N}\text{-}(CH_2)_2\text{-} \end{array}$$

wherein R, R_1 and R_2 independently have from 0 to 6 sites of unsaturation, and have the structure

$$CH_3\text{-}(CH_2)_a\text{-}(CH{=}CH\text{-}CH_2)_b\text{-}(CH_2)_c\text{-}Y$$

wherein the sum of a and c is from 1 to 23; and b is 0 to 6; and wherein Y is -C(O)O-, -C-O-, -C=C-O-, -C(O)S-, -C-S-, or -C=C-S-.

10. A liposome formed at least in part from a liponucleotide according to the formula of claim 6.

11. A pharmaceutical composition comprising a liponucleotide compound according to claim 6 and a pharmaceutically acceptable carrier.

12. A pharamceutical composition comprising a liponucleotide compound according to claim 6 and at least one other antiviral compound together with pharmaceutically acceptable carriers.

Patentansprüche

1. Verwendung einer Liponucleotidverbindung für die Herstellung einer Zusammensetzung zur Behandlung einer viralen oder retroviralen Infektion eines Säugers, welche umfasst,

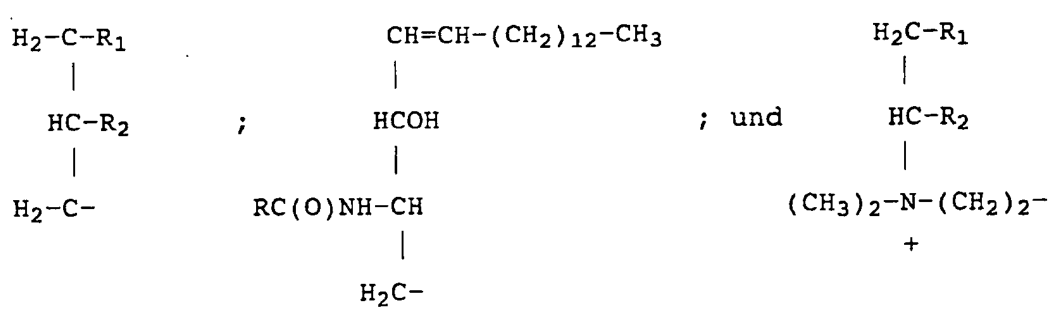
   ein Konjugat eines antiviralen Nucleosidanalogen, welches in dem zu behandelnden Säuger nicht natürlich vorkommt und welches durch die Fähigkeit gekennzeichnet ist, antivirale Aktivität gegenüber DNA-oder RNA-Viren zu zeigen, und eine mit der 5'-Position des Nucleosidanalogen verbundene Lipideinheit, wobei das Konjugat die Formel

   $$(L)_m\text{-}(W)_n\text{-}A\text{-}Q\text{-}Z$$

   besitzt, worin

   Z der Basenteil eines Nukleosidanalogen ist;
   Q ein Pentoserest oder ein azyklisches Fragment davon oder ein carbozyklisches Analoges ist;
   A O oder S ist;
   W Phosphat ist;
   N 1 bis 3 ist;

   Und L eine Lipideinheit ist, worin m = 1 bis 5 ist; worin L direkt an ein W gebunden ist und worin L ausgewählt ist aus



   worin R, R_1 und R_2 unabhängig voneinander an 0 bis 6 Positionen ungesättigt sind und die Struktur

   $$CH_3\text{-}(CH_2)_a\text{-}(CH{=}CH\text{-}CH_2)_b\text{-}(CH_2)_c\text{-}Y$$

   besitzen, worin die Summe von a und c 1 bis 23 beträgt; b 0 bis 6 ist und worin y -C(O)O-, -C-O-, C=C-O,-C(O)S-, -C-S-, oder - C=C-S- ist.

2. Verwendung nach Anspruch 1, wobei die nicht-natürlich vorkommende Nucleosidkomponente ein Analoges unter Modifizierung einer natürlich vorkommenden Base oder Pentose mittels Substitution, Deletion oder Austausch ist.

3. Verwendung nach Anspruch 1 oder 2, wobei der Pentoserest ein 2',3'-Deoxy, 2',3'Dideoxy, 2',3'Didehydro-, Azido- oder Halogen-Derivat von Ribose oder einem azyklischen hydroxylierten Fragment von Ribose ist.

4. Verwendung nach Ansprüchen 1 bis 3, worin die Gruppe -A-Q-Z ausgewählt ist aus der Gruppe bestehend aus

2',3'-Dideoxycytidin;
2',3'-Dideoxythymidin;
2',3'-Dideoxyguanosin;
2',3'-Dideoxyadenosin;
2',3'-Dideoxyinosin;
2,6-Diaminopurin-2',3'-dideoxyribosid;
2',3'-Dideoxy-2',3'-didehydrothymidin;
2',3'-Dideoxy-2',3'-didehydrocytidin carbozyklisch;
2',3'-Dideoxy-2',3'-diedehydroguanosin;
3'-Azido-3'-deoxythymidin;
3'-Azido-3'-deoxyguanosin;
2,6-Diaminopurin-3'-azido-2',3'-dideoxyribosid;
3'-Fluoro-3'-deoxythymidin;
3'-Fluoro-2',3'-dideoxyguanosin;
2',3'-dideoxy-2'-fluoro-ara-adenosin;
2,6-Diaminopurin-3'fluoro-2',3'-dideoxyribosid;
9-(4'-Hydroxy-1',2'-butadienyl)adenin;
3-(4'-Hydroxy-1',2'-butadienyl) cytosin;
9-(2-Phosphonylmethoxyethyl)adenin;
3-Phosphonomethoxyethyl-2,6-diaminopurin;
Acyclovir oder
Ganciclovir.

5. Verwendung nach einem der vorstehenden Ansprüche, worin die Nucleotidverbindung in einem Liposom vorliegt oder in der Lage ist, selbst ein Liposom zu bilden.

6. Ein antivirales oder antiretrovirales Liponucleotid der Formel

$$(L)_m\text{-}(W)_n\text{-}A\text{-}Q\text{-}Z$$

worin

Z der Basenteil eines Nucleosidanalogen ist,
Q ein Pentoserest oder ein azyklisches Fragment davon oder ein carbozyklisches Analoges ist;
A O oder S ist,
W Phosphat ist,
n 1 bis 3 ist
L eine Lipideinheit ist,
m 1 ist,

wobei die Lipideinheit ausgewählt ist aus

$$\begin{array}{l} H_2\text{-}C\text{-}R_1 \\ \quad | \\ HC\text{-}R_2 \\ \quad | \\ H_2\text{-}C\text{-} \end{array} \; ; \quad \begin{array}{l} CH{=}CH\text{-}(CH_2)_{12}\text{-}CH_3 \\ \quad | \\ HCOH \\ \quad | \\ RC(O)NH\text{-}CH \\ \quad | \\ H_2C\text{-} \end{array} \; ; \text{ und } \quad \begin{array}{c} H_2C\text{-}R_1 \\ | \\ HC\text{-}R_2 \\ | \\ (CH_3)_2\text{-}\overset{+}{N}\text{-}(CH_2)_2\text{-} \end{array}$$

worin R,R_1 und R_2 unabhängig voneinander an 0 bis 6 Positionen ungesättigt sind und die Struktur

$$CH_3\text{-}(CH_2)_a\text{-}(CH\text{=}CH\text{-}CH_2)_b\text{-}(CH_2)_c\text{-}Y$$

besitzen, worin die Summe von a und c 1 bis 23 beträgt; b 0 bis 6 ist und worin Y gleich -C(O)O-, -C-O-, C=C-O,-C(O)S, -C-S-, oder -C=C-S- ist,

mit der Maßgabe, dass die Verbindung in Form eines Liposoms vorliegt, wenn der Pentoserest Arabinofuranose und der Basenteil Cytosin oder Adenin ist,

mit der Maßgabe, dass, wenn das Lipid ein 1,2-Diradylglycerol ist und die Anbindung 3-Monophosphat ist, das Nukleosidanaloge nicht 5-Fluoruridine, 5-Fluorocytidin, Bredinin, Tubercidin, Arabinosyl-Cytosin, Arabinosyl-5-fluorocytosin, Arabinosyl-5-luorocytosin, Arabinosyl-Adenin, Arabinosyl-Thymin, 5-Fluoro-2'-Deoxyribouridin und Neplanocin A ist;

mit der Maßgabe, dass, wenn die Lipideinheit 1,2-Diradyl- oder 1,3-Diradylglycerol ist und die Anbindung Monophosphat ist, das Nukleosid nicht Cytidin, Uridin, Arabinosylcytosin, Adenosin, Guanosin, Cyclocytidin, Deoxyadenosin, Deoxycytidin, Deoxyguanosin, Deoxythymidin, Deoxyuridin oder Inosin ist;

mit der Maßgabe, dass, wenn die Lipideinheit 1,2-Di-O-Acylglycerol und die Anbindung 3-Diphosphat ist, das Nukleosid nicht Cytidin, Arabinosylcytosin, Arabinosyladenin, 7-Deazaadenosin ist; vorausgesetzt dass, wenn die Lipideinheit 1,2-Di-O-Alkylglycerol ist und die Anbindung 3-Diphosphat ist, das Nukleosid nicht Arabinosylcytosin ist; vorausgesetzt dass, wenn die Lipideinheit 1,2-Di-O-Acylglycerol und die Anbindung Monophosphat ist, das Nukleosid nicht Arabinosylcytosin ist;

und unter der Maßgabe, dass, wenn die Lipideinheit 1-O-Alkyl-2-O-alcylglycerol ist, der Zucker Ribose, 2'-Deoxyribose, Arabinose oder 2'2'-Dihydroxyribose und die Anbindung Diphosphat ist, das Nukleosid nicht als Base Adenin, Cytosin, 5-Fluorouracil, 5-Azacytosin, 6-Mercaptopurin oder 7-Deazaadenin enthält.

7. Eine Verbindung gemäß Anspruch 6, worin die Gruppe A-Q-Z ausgewählt ist aus den Nukleosiden, die umfassen

2',3'-Dideoxycytidin;
2',3'-Dideoxythymidin;
2',3'-Dideoxyguanosin;
2',3'-Dideoxyadenosin;
2',3'-Dideoxyinosin;
2,6-Diaminopurin-2',3'-dideoxyribosid;
2',3'-Dideoxy-2',3'-didehydrothymidin;
2',3'-Dideoxy-2',3'-didehydrocytidin carbozyklisch;
2',3'-Dideoxy-2',3'-diedehydroguanosin;
3'-Azido-3'-deoxythymidin;
3'-Azido-3'-deoxyguanosin;
2,6-Diaminopurin-3'-azido-2',3'-dideoxyribosid;
3'-Fluoro-3'-deoxythymidin;
3'-Fluoro-2',3'-dideoxyguanosin;
2',3'-dideoxy-2'-fluoro-ara-adenosin;
2,6-Diaminopurin-3'fluoro-2',3'-dideoxyribosid;
9-(4'-Hydroxy-1',2'-butadienyl)adenin;
3-(4'-Hydroxy-1',2'-butadienyl) cytosin;
9-(2-Phosphonylmethoxyethyl)adenin;
3-Phosphonomethoxyethyl-2,6-diaminopurin;
Acyclovir oder
Ganciclovir.

8. Verbindungen nach Anspruch 6, ausgewählt aus der Gruppe bestehend aus

Phosphatidyl(3'-azido-3'-deoxy)thymidin (pAZT);
Phosphatidyl(2',3'-dideoxy)cytidin(pddC);

Phosphatidyl(2',3'-dideoxy)thymidin(pddT);
(3'-Azido-3'-deoxy)thymidin-diphosphatdiglycerid (AZTdpdg);
Phosphatidylacyclovir (pACV);
1-O-Stearylglycero-rac-3-phospho-5'-(3'azido,3'-deoxy)thymidin.

9. Liposom, welches wenigsten teilweise aus einer Liponukleotidverbindung gebildet wird, die umfasst:

ein Konjugat eines antiviralen Nukleosidanalogen, welches nicht natürlich in dem zu behandelnden Säuger auftritt und welches durch die Fähigkeit, antivirale Aktivität gegenüber DNA- oder RNA Viren zu zeigen charakterisiert ist, und eine Lipideinheit, verbunden mit der 5'-Position des Nukleosidanalogen, wobei das Konjugat die Formel

$$(L)_m\text{-}(W)_n\text{-}A\text{-}Q\text{-}Z$$

besitzt, worin

Z der Basenteil eines Nukleosidanalogen ist,
Q ein Pentoserest oder ein azyklisches Fragment davon oder ein carbozyklisches Analoges ist;
A O oder S ist;
W Phosphat ist;
n 1 bis 3 ist

und L eine Lipideinheit ist, worin m = 1 bis 5 ist; worin L direkt an ein W gebunden ist und worin L ausgewählt ist aus

$$\begin{array}{c} H_2\text{-}C\text{-}R_1 \\ | \\ HC\text{-}R_2 \\ | \\ H_2\text{-}C\text{-} \end{array} \quad ; \quad \begin{array}{c} CH{=}CH\text{-}(CH_2)_{12}\text{-}CH_3 \\ | \\ HCOH \\ | \\ RC(O)NH\text{-}CH \\ | \\ H_2C\text{-} \end{array} \quad ; \text{ und } \quad \begin{array}{c} H_2C\text{-}R_1 \\ | \\ HC\text{-}R_2 \\ | \\ (CH_3)_2\text{-}\overset{}{\underset{+}{N}}\text{-}(CH_2)_2\text{-} \end{array}$$

worin R,R_1 und R_2 unabhängig voneinander an 0 bis 6 Positionen ungesättigt sind und die Struktur

$$CH_3\text{-}(CH_2)_a\text{-}(CH{=}CH\text{-}CH_2)_b\text{-}(CH_2)_c\text{-}Y$$

besitzen, worin die Summe von a und c 1 bis 23 beträgt; b 0 bis 6 ist und worin Y -C(O)O-, -C-O-, -C=C-O-, -C(O)S-, -C-S-, oder -C=C-S- ist.

10. Liposom, wenigsten teilweise gebildet aus einem Liponukleotid gemäß der Formel aus Anspruch 6.

11. Pharmazeutische Zusammensetzung, die eine Liponukleotidverbindung gemäß Anspruch 6 und einen pharmazeutisch vertretbaren Träger umfaßt.

12. Pharmazeutische Zusammensetzung, die eine Liponukleotidverbindung nach Anspruch 6 und wenigstens eine weitere antivirale Verbindung zuammen mit pharmazeutisch vertretbaren Trägern umfasst.

Revendications

1. Utilisation pour la préparation d'une composition de traitement d'une infection virale ou rétrovirale chez le mammifère, d'un liponucléotide comprenant

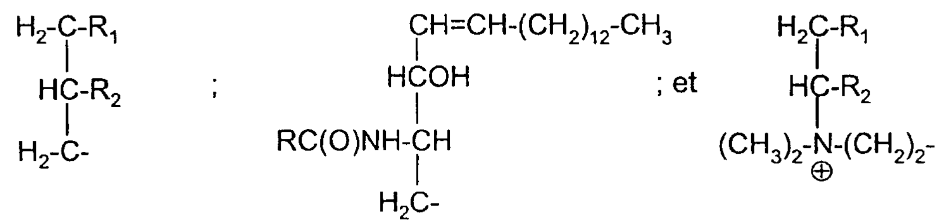
un conjugué d'un analogue antiviral de nucléoside qui ne se produit pas naturellement chez le mammifère à traiter et qui est caractérisé par l'aptitude à exercer une activité antivirale sur les virus d'ADN ou d'ARN, et un radical de lipide relié en la position 5' de l'analogue de nucléoside
dans lequel le conjugué a la formule

$$(L)_m\text{-}(W)_n\text{-}A\text{-}Q\text{-}Z$$

dans laquelle

- Z est la partie de base d'un analogue de nucléoside ;
- Q est un résidu pentosique ou son fragment acyclique ou un analogue carbocyclique ;
- A est O ou S ;
- W est un phosphate ;
- n est 1 à 3 ;

et L est un radical de lipide dans laquelle m = 1 à 5 ; L étant relié directement à un W, et dans laquelle L est choisi parmi



dans lesquelles R, R_1 et R_2 ont indépendamment de 0 à 6 sites d'insaturation et ont la structure

$$CH_3\text{-}(CH_2)_a\text{-}(CH{=}CH\text{-}CH_2)_b\text{-}(CH_2)_c\text{-}Y$$

dans laquelle la somme de a et c est comprise entre 1 et 23 ; et b est compris entre 0 et 6 ; et dans laquelle Y est -C(O)O-, -C-O-, -C=C-O-, -C(O)S-, -C-S-, ou -C=C-S-.

2. Utilisation suivant la revendication 1, dans laquelle le constituant de nucléoside qui ne se produit pas naturellement est un analogue avec modification d'une base naturelle ou d'un pentose naturel par substitution, suppression ou remplacement.

3. Utilisation suivant la revendication 1 ou 2, dans laquelle le résidu pentosique est un dérivé 2',3'-désoxy-2',3'-didésoxy-2',3'-didéhydro, azido ou halogéno de ribose ou un fragment acyclique hydroxylé de ribose.

4. Utilisation suivant la revendication 1 à 3, dans laquelle le groupe -A-Q-Z est choisi dans le groupe consistant en les

2',3'-didésoxycytidine ;
2',3'-didésoxythymidine ;
2',3'-didésoxyguanosine ;
2',3'-didésoxyadénosine ;
2',3'-didésoxyinosine ;
2,6-diaminopurine-2',3'-didésoxyriboside ;
2',3'-didésoxy-2',3'-didéhydrothymidine ;
2',3'-didésoxy-2',3'-didéhydrocytidine carbocylique ;
2',3'-didésoxy-2',3'-didéhydroguanosine ;
3'-azido-3'-désoxythymidine ;
3'-azido-3'-désoxyguanosine ;
2,6-diaminopurine-3'-azido-2',3'-didésoxyriboside ;
3'-fluoro-3'-didésoxythimidine ;
3'-fluoro-2',3'-didésoxyguanosine ;

2',3'-didésoxy-2'-fluoro-ara-adénosine ;
2,6-diaminopurine-3'-fluoro-2',3'-didésoxyriboside ;
9-(4'-hydroxy-1',2'-butadiényl)adénine ;
3-(4'-hydroxy-1',2'-butadiényl)cytosine ;
9-(2-phosphonylméthoxyéthyl)adénine ;
3-phosphonométhoxyéthyl-2,6-diaminopurine ;
acyclovir ou
ganciclovir.

5. Utilisation suivant l'une quelconque des revendications précédentes, dans laquelle le nucléotide est présent dans un liposome ou est capable de former de soi-même un liposome.

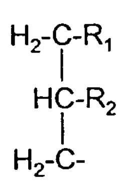
6. Liponucléotide antiviral ou antirétroviral de formule

$$(L)_m\text{-}(W)_n\text{-}A\text{-}Q\text{-}Z$$

dans laquelle

Z est la partie de base d'un analogue de nucléoside ;
Q est le résidu pentosique ou son fragment acyclique ou un analogue carboxyclique ;
A est O ou S ;
W est un phosphate ;
n est 1 à 3 ;
L est un radical de lipide ;
m est 1

dans laquelle le radical de lipide est choisi parmi



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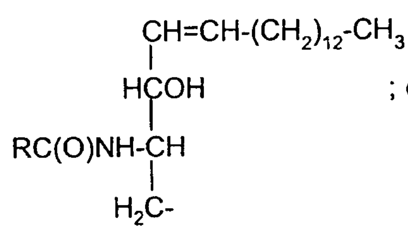
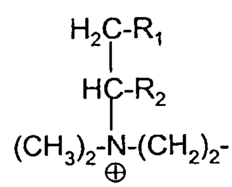


; et



dans lesquelles R, R_1 et R_2 ont indépendamment de 0 à 6 sites d'insaturation et ont la structure

$$CH_3\text{-}(CH_2)_a\text{-}(CH{=}CH\text{-}CH_2)_b\text{-}(CH_2)_c\text{-}Y$$

dans laquelle la somme de a et c est comprise entre 1 et 23 ; et b est compris entre 0 et 6 ; et dans laquelle Y est -C(O)O-, -C-O-, -C=C-O-, -C(O)S-, -C-S-, ou -C=C-S-,

sous réserve que le composé soit sous la forme d'un liposome lorsque le résidu pentosique est un arabinofuranose et lorsque la partie de base est la cytosine ou l'adénine,
sous réserve que lorsque le lipide est un 1,2-diradylglycérol et la liaison est un 3-monophosphate, l'analogue de nucléoside n'est pas la 5-fluoro-uridine, la 5-fluorocytidine, la brédinine, la tubercidine, l'arabinosylcytosine, l'arabinosyl-5-fluorocytosine, l'arabinosyladénine, l'arabinosyl-thymine, la 5-fluoro-2'-désoxyribo-uridine et la néplanocine A ;
sous réserve que lorsque le radical de lipide est un 1,2-diradylglycérol ou un 1,3-diradylglycérol et la liaison est un monophosphate, le nucléoside n'est pas la cytidine, l'uridine, l'arabinosylcytosine, l'adénosine, la guanosine, la cyclocitidine, la désoxyadénosine, la désoxycytidine, la désoxyguanosine, la désoxythymidine, la désoxyuridine ou l'inosine ;
sous réserve que lorsque le radical de lipide est un 1,2-di-O-acylglycérol et la liaison est un 3-diphosphate, le nucléoside n'est pas la cytidine, l'arabinosylcytosine, l'arabinosyladénine, la 7-déazaadénosine ; pourvu que

lorsque le radical de lipide est un 1,2-di-O-alcoylglycérol et la liaison est un 3-diphosphate, le nucléoside ne soit pas une arabinosylcytosine ; pourvu que lorsque le radical de lipide est un 1,2-di-O-acylglycérol et la liaison est un monophosphate, le nucléoside ne soit pas un arabinosylcytosine ;

et sous réserve que lorsque le radical de lipide est un 1-O-alcoyl-2-O-acylglycérol, le sucre est le ribose, le 2'-désoxyribose, l'arabinose ou le 2,2'-dihydroxyribose et la liaison est un diphosphate, le nucléoside ne contient pas une base qui est l'adénine, la cytosine, la 5-fluoro-uracile, la 5-azacytosine, la 6-mercaptopurine ou la 7-déazaadénine.

7. Composé suivant la revendication 6, dans laquelle le groupe A-Q-Z est choisi parmi les nucléosides comprenant

la 2',3'-didésoxycytidine ;
la 2',3'-didésoxythymidine ;
la 2',3'-didésoxyguanosine ;
la 2',3'-didésoxyadénosine ;
la 2',3'-didésoxyinosine ;
la 2,6-diaminopurine-2',3'-didésoxyriboside ;
la 2',3'-didésoxy-2',3'-didéhydrothymidine ;
le 2',3'-didésoxy-2',3'-didéhydrocytidine carbocylique ;
la 2',3'-didésoxy-2',3'-didéhydroguanosine ;
la 3'-azido-3'-désoxythymidine ;
la 3'-azido-3'-désoxyguanosine ;
le 2,6-diaminopurine-3'-azido-2',3'-didésoxyriboside ;
la 3'-fluoro-3'-désoxythimidine ;
la 3'-fluoro-2',3'-didésoxyguanosine ;
la 2',3'-didésoxy-2'-fluoro-ara-adénosine ;
le 2,6-diaminopurine-3'-fluoro-2',3'-didésoxyriboside ;
la 9-(4'-hydroxy-1',2'-butadiényl)adénine ;
la 3-(4'-hydroxy-1',2'-butadiényl)cytosine ;
la 9-(2-phosphonylméthoxyéthyl)adénine ;
la 3-phosphonométhoxyéthyl-2,6-diaminopurine ;
l'acyclovir ou
le ganciclovir.

8. Composés suivant la revendication 6, choisis dans le groupe consistant en

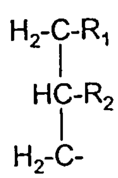
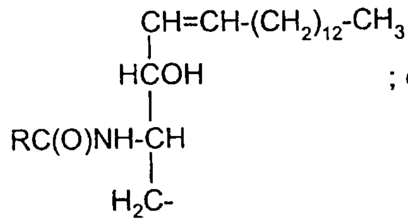
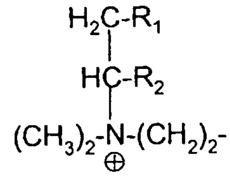
la phosphatidyl(3'-azido-3'-désoxy)thymidine (pAZT) ;
la phosphatidyl(2',3'-didésoxy)cytidine (pddC) ;
la phosphatidyl(2',3'-didésoxy)thymidine (pddT) ;
la (3'-azido-3'-désoxy)thymidine diphosphate diglycéride (AZTdpdg) ;
la phosphatidylacyclovir (pACV) ;
la 1-O-stéarylglycéro-rac-3-phospho-5'-(3'azido,3'-désoxy)thymidine.

9. Liposome formé au moins en partie d'un liponucléotide comprenant

un conjugué d'un analogue antiviral de nucléoside qui ne se produit pas naturellement chez le mammifère à traiter et qui est caractérisé par l'aptitude à exercer une activité antivirale sur les virus d'ADN ou d'ARN, et un radical de lipide relié en la position 5' de l'analogue de nucléoside
dans lequel le composé a la formule dans laquelle

Z est la partie de base d'un analogue de nucléoside ;
Q est un résidu pentosique ou son fragment acyclique ou un analogue carbocyclique ;
A est O ou S ;
W est un phosphate ;
n est 1 à 3 ;

et L est un radical de lipide dans laquelle m = 1 à 5 ; L étant relié directement à un W, et dans laquelle L est choisi parmi

 ;  ; et 

dans lesquelles R, R_1 et R_2 ont indépendamment de 0 à 6 sites d'insaturation et ont la structure

$$CH_3\text{-}(CH_2)_a\text{-}(CH{=}CH\text{-}CH_2)_b\text{-}(CH_2)_c\text{-}Y$$

dans laquelle la somme de a et c est comprise entre 1 et 23 ; et b est compris entre 0 et 6 ; et dans laquelle Y est -C(O)O-, -C-O-, -C=C-O-, -C(O)S-, -C-S-, ou -C=C-S-.

10. Liposome formé au moins en partie d'un liponucléotide suivant la formule de la revendication 6.

11. Composition pharmaceutique comprenant un liponucléotide suivant la revendication 6 et un véhicule acceptable pharmaceutiquement.

12. Composition pharmaceutique comprenant un liponucléotide suivant la revendication 6 et au moins un autre composé antiviral associés à des véhicules acceptables pharmaceutiquement.

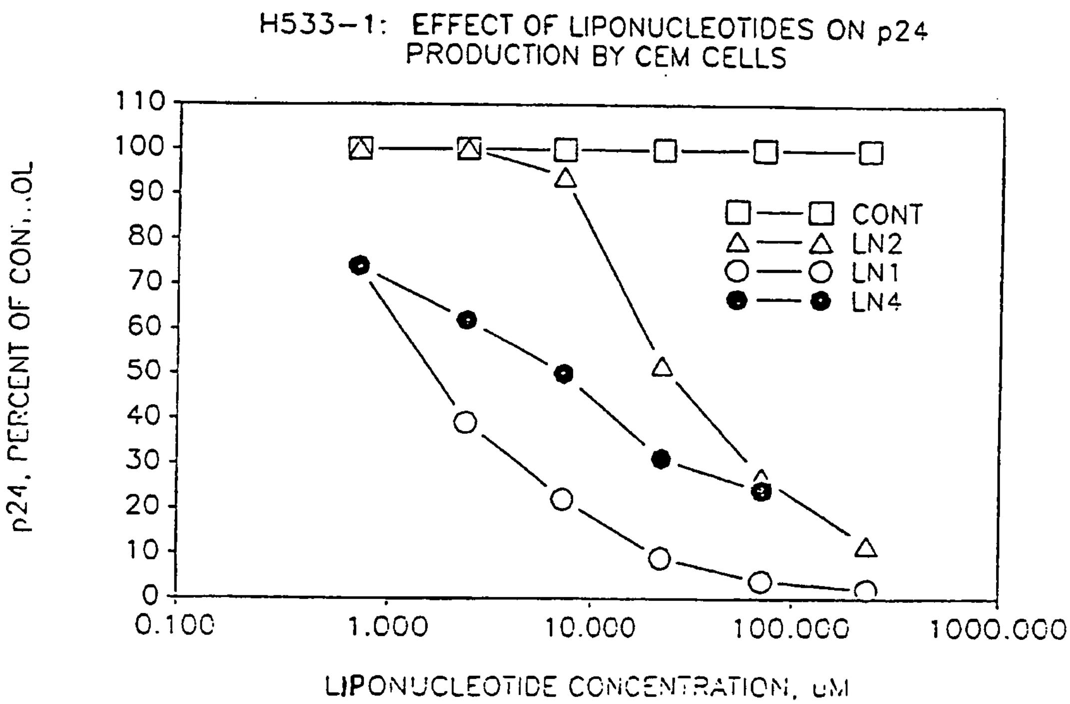

H533-1: EFFECT OF LIPONUCLEOTIDES ON p24
PRODUCTION BY CEM CELLS
110
100
90
80
70
60
50
40
30
20
10
0
p24, PERCENT OF CONT..OL
CONT
LN2
LN1
LN4
0.100
1.000
10.000
100.000
1000.000
LIPONUCLEOTIDE CONCENTRATION, uM

FIGURE 1

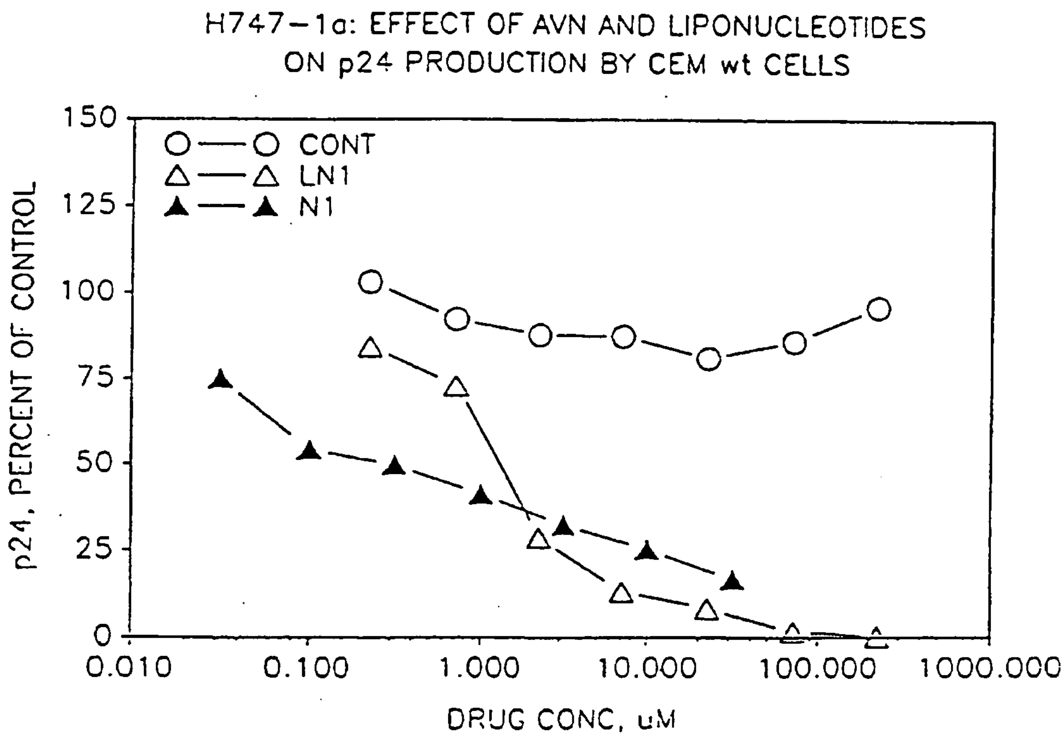

H747-1a: EFFECT OF AVN AND LIPONUCLEOTIDES
ON p24 PRODUCTION BY CEM wt CELLS
150
125
100
75
50
25
0
p24, PERCENT OF CONTROL
CONT
LN1
N1
0.010
0.100
1.000
10.000
100.000
1000.000
DRUG CONC, uM

FIGURE 2

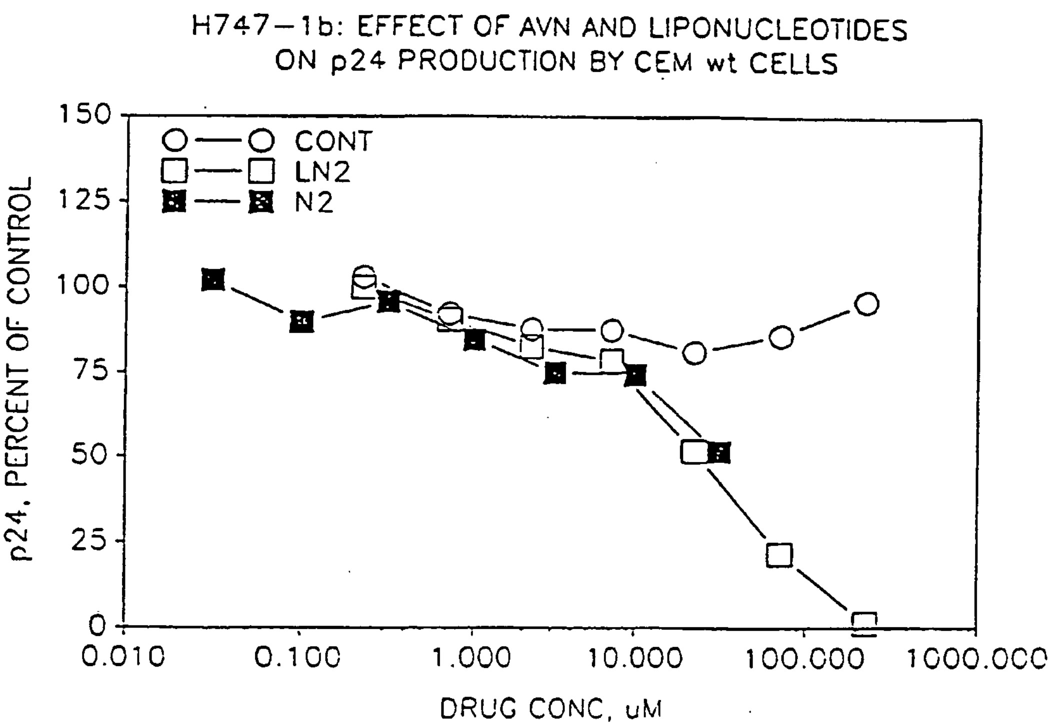

H747−1b: EFFECT OF AVN AND LIPONUCLEOTIDES
ON p24 PRODUCTION BY CEM wt CELLS
CONT
LN2
N2
p24, PERCENT OF CONTROL
0
25
50
75
100
125
150
0.010
0.100
1.000
10.000
100.000
1000.000
DRUG CONC, uM

FIGURE 3

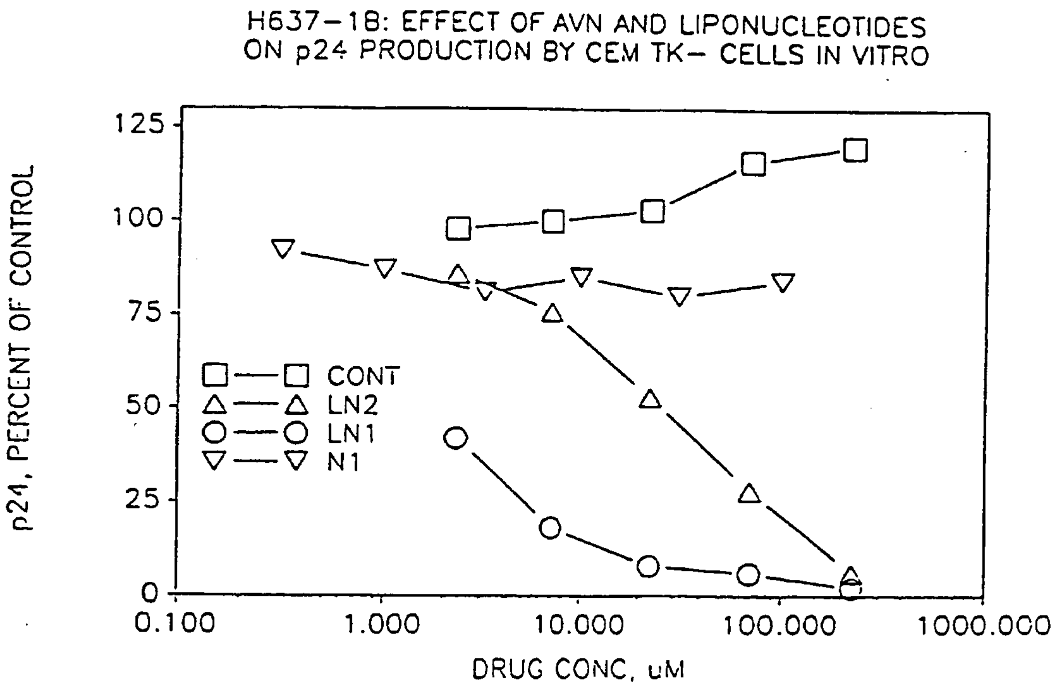

H637-18: EFFECT OF AVN AND LIPONUCLEOTIDES
ON p24 PRODUCTION BY CEM TK- CELLS IN VITRO
p24, PERCENT OF CONTROL
125
100
75
50
25
0
0.100
1.000
10.000
100.000
1000.000
DRUG CONC, uM
CONT
LN2
LN1
N1

FIGURE 4

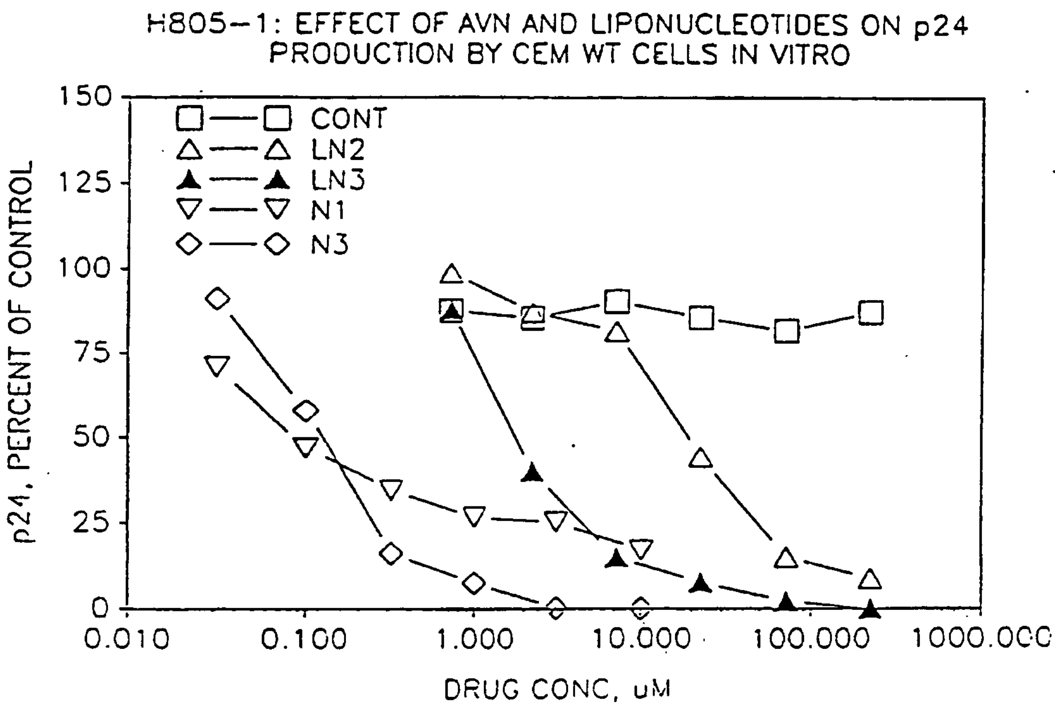

H805-1: EFFECT OF AVN AND LIPONUCLEOTIDES ON p24
PRODUCTION BY CEM WT CELLS IN VITRO
CONT
LN2
LN3
N1
N3
p24, PERCENT OF CONTROL
150
125
100
75
50
25
0
0.010
0.100
1.000
10.000
100.000
1000.000
DRUG CONC, uM

FIGURE 5